THE
JOURNAL OF
COMPARATIVE PATHOLOGY
AND
THERAPEUTICS.

Vol. XLVI.—No. 3. SEPTEMBER 30th, 1933. PRICE 4s.

STUDIES ON BOVINE MASTITIS—VIII. THE CONTROL OF CHRONIC STREPTOCOCCUS MASTITIS.

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PREVIOUS papers in this series of publications have treated of the bacteriology of streptococcus mastitis and of the diagnosis of the prevalent chronic form of the disease. The streptococci which cause this form have been referred to by us as Group I mastitis streptococci and they correspond to the organism often described as *Streptococcus mastitidis contagiosæ*, *Streptococcus agalactiæ*, or in the literature of Central Europe as the "Galtstreptokokkus." As we have pointed out, some Group I strains produce beta-hæmolytic colonies when growing in deep ox-blood agar plates, while with others the colonies are non-hæmolytic and slightly greenish. During the present century an extensive literature has grown up on the subject of mastitis due to *S. agalactiæ*; in fact, this organism has been the main concern of investigators. It is now recognised, however, that mastitis is sometimes caused by other kinds of streptococci. Thus, strongly hæmolytic streptococci, bearing a close resemblance to *S. pyogenes* of man, may invade the udder and set up a clinically severe infection.

In 1929 we pointed out that bovine mastitis may be caused by non-hæmolytic streptococci other than those of Group I, and certain of these were relegated to a Group II. Diernhofer (1930) also referred to cases where streptococci other than *S. agalactiæ* were the cause of mastitis, and the characters of some of these strains were similar to those of our Group II. S. J. Edwards (1932) reported on the examination of a large series of Group II strains, and also referred to mastitis streptococci which were placed in yet another group—Group III. Diernhofer (1932), having examined several of our strains, states quite definitely that certain of the strains described by

him in 1930 were identical with those belonging to Group II. These he proposes to name *S. dysgalactiæ*, while the term *S. uberis* is applied to organisms with the characters of our Group III. On the whole, the pathological changes caused by Group II streptococci tend to follow a more acute course than those of Group I and some cases may be extremely severe and lead to speedy destruction of the involved quarter. With Group III streptococci the changes are generally mild and may be transient or of a somewhat chronic nature and produce only slight alterations in the milk. It must be made clear, however, that apart perhaps from selected herds, Groups II and III infections are of much smaller economic importance than those due to Group I. The former appear to arise sporadically—in fact on one occasion a Group II infection was observed prior to first calving —and there is no evidence that they spread by contagion. On the other hand, it is quite generally agreed that the chronic infections due to Group I are exogenous and are distributed during the course of milking which in most herds is practised indiscriminately. The reasons for this belief are as follows.

The streptococcus of chronic mastitis is regarded as an obligatory parasite, since it fails to multiply at low temperatures, *e.g.* 10° C., under natural conditions is only found in the udder and its secretion, and such information as is available suggests that its resistance outside the body is not great. Heifers at first calving are free from infection and the incidence of the disease rises with age when animals are exposed to infection. According to Seelemann (1932), in herds in which the amount of streptococcus mastitis is very small fresh cases are not likely to occur unless infected cows are added to the herd. It is the rule for fresh infections to appear when infected and healthy cows stand side by side, whereas when infected cows are stalled and milked separately the incidence of fresh infection is strikingly reduced or may be completely arrested. Theoretically, therefore, it should be possible to eliminate this form of mastitis by keeping healthy and infected cows apart, but in the literature on the subject no indication has been found that elimination has been achieved. For instance, in the comprehensive monographs by Klimmer and Haupt (1930) and by Rosell (1933) no mention can be found of actual instances where streptococcus mastitis has been eliminated from a herd by preventive measures which include separate milking of infected cows, though Klimmer and Haupt refer to an experiment of this nature which they are conducting.

The importance of streptococcus mastitis and the need for conducting an organised campaign against it have been emphasised by Seelemann, of the Dairy Research Institute at Kiel, and he himself has made serious efforts in control work. In his monograph published in 1932 Seelemann gave information regarding two herds (K and Re), in which all infected cows, after having been located by laboratory diagnostic methods, including surface plating on a plain agar medium, were stalled and milked separately and also kept apart from the healthy ones at pasture. It is reported that in Herd K (155 cows) between November, 1929, and February, 1931, the incidence of infection was reduced from 40 per cent. to about 10 per cent., and in Herd Re (134 cows) between February, 1929, and March, 1931, infection was reduced from 70 per cent. to 20 per cent. Actually, in both cases, the lowered incidence was partly due to the sale of infected animals. Seelemann is now

making similar observations in other herds, but, in addition, the infected cows are now receiving chemo-therapeutic treatment consisting of udder infusions with the acridine derivatives known as 'rivanol' or 'entozon.' In this way the incidence of true Galt infections in six large herds has been greatly reduced, but the disease has not yet been eliminated. A brief outline of progress in these herds is reported in a quite recent publication (Seelemann, 1933[1]), and from this it may be observed incidentally that in herd Re just referred to the incidence of infection by March, 1933, had been reduced to 4·2 per cent.

Seelemann (1933) concludes that by adopting hygienic control measures against mastitis great improvements can be effected and the appearance of fresh cases can in time be prevented. On the other hand, he considers that no one is yet in a position to say whether such an improvement will be permanent or whether fresh infections will break out. The need for prolonged and intensive investigation on the subject is recognised, but, as both Steck (1932) and Seelemann (1932) point out, the task of control is not as simple as might perhaps be imagined, and close collaboration is necessary between those who are doing the diagnostic laboratory work and those in charge of the herd.

The conclusion reached from a survey of the literature is that, while some efforts, and particularly those of Seelemann, appear to be full of promise, no one up to the present has brought decisive proof that chronic streptococcus mastitis can be eliminated from a herd by separating infected cows and having them milked last. The reason for this is readily understood when one bears in mind the insidious character of the disease, the amount of labour that may be involved in repeated examination of milk samples, as well as circumstances beyond the control of the investigator, such as the impossibility of constant supervision and the chances of error on the part of the milkers.

We have never regarded such difficulties as insurmountable obstacles in attempts at controlling the disease, especially as, in the present state of knowledge, there is no other satisfactory way of dealing with the problem. Since 1929, one of our main objects has, therefore, been to find out to what extent it is possible to control or eliminate chronic streptococcus mastitis by the theoretically simple procedure of picking out infected cows and having them milked last. The investigations have been carried out in seven herds comprising at the start some 550 cows. The " clean " portions of these herds have been examined at approximately three-monthly intervals, samples of milk from individual quarters of each cow having been tested in the laboratory for the presence of streptococci, with particular reference to those belonging to Group I. It is not proposed to deal with the general progress of this work here, beyond making the bare statement that one herd—Herd I—appears to have been free from infection since July, 1932, a result to be attributed largely to extensive selling of infected cows. The main purpose of this paper is to describe the events in Herd B, which, we believe, illustrate for the first time the fact that it is possible to assemble and maintain for at least three years a group of cows free from Group I streptococci.

History of Herd B.

Technique used in the Examination of Milk Samples.—After the udder had been wiped with a damp cloth, one or two streams of the fore milk were rejected, and then 15 to 20 c.c. milk from each quarter was taken into separate sterile tubes, which were immediately corked. The samples were then cooled and despatched by train to the Institute as soon as convenient. On arrival they were stored in a cool place and the examination was made the following day. After the reaction of the milk had been taken with brom-cresol-purple paper, 10 c.c. amounts were centrifuged for five minutes at 2,000 r.p.m. and the amount and character of the deposit noted. The deposit was then mixed with about 1·0 c.c. saline, of which one half was transferred to a Petri dish and the other half mixed with 9·0 c.c. sterile saline and 1·0 c.c. of the dilution transferred to a second Petri dish. Into each dish was poured about 10 c.c. agar, containing 5 per cent. defibrinated ox blood ; the contents were mixed and the plates incubated at 37° C. for 48 hours. After 24 and 48 hours suspicious colonies were picked to tubes of broth containing 1 per cent. horse serum and streptococcus growths were tested for their action upon lactose, saccharose, salicin, mannite, inulin, raffinose, sodium hippurate, and for their behaviour towards milk containing 1 : 20,000 methylene blue. Since April, 1930, an enriching medium has also been used, consisting of broth containing 5 per cent. horse serum and 1 per cent. glucose, to which was added crystal violet* to a final concentration of 1 : 200,000 (Edwards, 1930). The crystal violet inhibits the growth of many of the organisms normally present in milk, in particular staphylococci, without interfering with the development of streptococci. If streptococci appear in this medium their nature is determined by plating in blood agar and by transferring two representative colonies to test media for identification. As a further control, during the past two years nearly all the streptococcus strains (about 250 in number) isolated at various times from milk samples of this herd have been checked serologically by Stableforth according to the methods he has outlined (1932).

Herd B is made up of pedigree animals of the Red Poll breed. It was formed in 1914 and has been built up almost entirely from its own stock and during the five years' period of observation, from June, 1928, only four animals have been purchased, *viz.*, in July, 1929, three maiden heifers and one heifer in the first pregnancy. The history suggests remarkable freedom from tuberculosis and contagious abortion, though no complete herd tests have been carried out. Milking has always been by hand and the men are instructed to wash their hands before milking each cow. Every precaution has also been taken with the milking of cows suspected of udder disease. When first examined in June, 1928, the herd contained 53 adult females (cows in milk, dry cows and heifers due to calve). Milk samples were available from 49 of these animals from June to December, 1928, and of these ten were found to be infected with Group I mastitis streptococci ; two other cows which could not be freed from suspicion were also placed with the infected group (see Table I). This is not as high a proportion as has often been observed and it is probable that the good supervision and the prompt removal

* Product from T. G. Gurr, 136, New King's Road, London, S.W.6.

of cows giving altered milk for use as nurse animals prevented the incidence rising higher. Of the 49 cows from which milk samples were examined in 1928, 22 were first calvers, 7 were second calvers, 10 were third and fourth calvers, and 10 had had from 5 to 9 calves. Of the 12 cows placed in the infected group, 7 had had 6 to 8 calves, 4 were third or fourth calvers and 1 had had 2 calves. For the purpose of comparison, the constitution of the herd in June, 1933, was: 9 first calvers, 7 second calvers, 9 third or fourth calvers and 7 which had had from 5 to 8 calves.

As soon as a preliminary diagnosis has been made, it was requested that the infected cows should remain on the same premises (X) and be allowed to use the same pastures, but should always be milked last. This practice was followed until September, 1930, when the herd was divided, the few infected cows which remained being moved to another lot of premises (Y) and used for suckling calves, and the healthy portion to premises Z. Henceforward, all calf-rearing was carried out on premises Y and no cow, mastitis-infected or otherwise, which was moved from Z to Y has ever been moved back again. Further, it is considered that the risk of infection being transferred from premises Y to Z by human agency has been very remote. Between January, 1929, and June, 1933, milk samples from the individual quarters of the " clean " portion of the herd have been examined on 17 occasions at intervals of from three to five months and, in addition, samples have been examined from cows shortly after calving and from any about which there was suspicion.

TABLE I.

Date of Examination.	*Number of cows of which the milk was examined.*	*Result.*		
		Healthy.	*Infected.*	*Doubtful.*
1928, June to December	49	37	10	2
1929 ...	39	37	2	0
1930 ...	36	36	0	0
1931 ...	27	27	0	0
1932 ...	32	32	0	0
1933, Jan. to June ...	32	32	0	0

Note.—After December, 1928, the " clean " portion only of the herd was examined as a routine.

Table I shows for each year from June, 1928, to June, 1933, the number of cows of which the milk was examined and the number of healthy, infected and doubtful. From this it will be seen that no fresh cases of Group I infection have appeared since the beginning of 1930. It is also important to mention the number of lactations completed by cows which formed part of the herd during the five years' period of observation and which remained healthy throughout their stay. The total number of such animals was 70. Of these,

14 did not complete one lactation ; 27 completed one lactation ; 16, two lactations ; 7, three lactations, while 6 cows passed through four complete lactations. It is hoped to continue these observations for a longer time still.

The 10 originally infected cows were among the oldest in the herd ; one of them was infected in one quarter ; four in two quarters ; four in three quarters, and one in four quarters ; two of them had only three quarters each in June, 1928. In all these cases the streptococci were present in the milk in large numbers from time to time and produced narrow beta-hæmolytic zones around the colonies in deep ox-blood agar plates. A number of these cows had shown clots in the milk and more obvious symptoms of mastitis when drying or after calving. Six of the ten cows were still excreting beta-hæmolytic streptococci in their milk at the final test, carried out between two and seven months before they left the herd. The other four animals remained on the farm for not less than two years and they appeared to have recovered, since beta-hæmolytic streptococci gradually disappeared from their milk and the secretion eventually became quite normal in character. The last occasion on which beta-hæmolytic streptococci were isolated from the milk of any of these four animals was in March, 1931. During 1929 two other cows were added to the infected group, as they were showing some disturbance in the milk and shedding streptococci with reactions of Group I organisms. These were old cows and each of them had lost a quarter previously, in one case owing to infection with *Fusiformis necrophorus*. By June, 1933, only one of the originally infected cows remained on the farm, and this one also had apparently recovered since July, 1930.

Reference was made to Herd B in an article dealing with the more acute forms of streptococcus mastitis, published by us in 1932. The absence of Group I streptococci from this herd has not caused the disappearance of udder troubles—in fact the herd appears to have been exceptionally unfortunate in this respect. Thus, since June, 1928, there are records of mastitis due to *C. pyogenes* in nine cows, and in one instance the uterus also was infected with this organism, which was apparently responsible for the death of the calf. There have also been two cases of mastitis due to *Bact. coli*, one due to *F. necrophorus* and one apparently caused by yeasts. Streptococci belonging to mastitis Groups II and III have been isolated from the milk of some 18 cows and on numerous occasions during the last five years. These organisms were sometimes associated with changes in the character of the milk, which were either obvious to the owner or only found on laboratory examination ; at other times similar changes were present but no suspicious organisms could be isolated. One case in particular should be mentioned. Thie was an animal which, just before her first calving, was noticed to havs a swollen quarter. Some watery fluid containing clots was drawn off, and bacteriological examination showed Group II mastitis streptococci in very large numbers and in apparently pure culture.

Discussion.

The literature which has accumulated on the subject of bovine mastitis is now very large, but we are not aware that anyone previously has proved that a herd of cows can be built up and maintained for several years free from the streptococci commonly associated with chronic mastitis (Group I mastitis streptococci). We believe that in Herd B we have succeeded in doing this, for the following reason. On 12 occasions between January, 1930, and June, 1933, milk samples from the individual quarters of all cows have been examined by a technique proved in other herds to be capable of detecting very small numbers of Group I mastitis streptococci, and in no instance have these organisms been found. On the other hand, mastitis streptococci of Groups II and III have been isolated very many times, both from cows in which, at the time, they were apparently responsible for disturbance and also quite often from cows showing no disturbance. We, therefore, think that we have provided the final proof that chronic streptococcus mastitis is a strictly contagious disease, in the same way that bovine tuberculosis, contagious abortion and Johne's disease are generally accepted to be. The result in Herd B has been made possible largely because the conditions were ideal for scientific observation and because the owner and his staff took a personal interest in the investigations.

Although chronic streptococcus mastitis is very widespread, it is quite likely that herds free from the disease do exist. Rolle (1932), for instance, says that he has not met with *S. agalactiæ* mastitis in Latvia, and that there is very little of it in the northerly regions in the vicinity or in East Prussia. Seelemann (1932) says that he is acquainted with a number of herds which have remained free from " Galt " for considerable periods. No details are given in his publication, and, without several complete herd tests with a searching technique, one could not be sure that the herds in question were really free from latent infections.

Herd B is regarded as of great value as a demonstration and the example will serve as a firm basis for comparison in further efforts. Apart from this, and apart from the economic aspect, it would be useful to build up other similar herds, as information could then be obtained on a number of points : (1) Such herds would provide a clear background for the study of other forms of mastitis to which the cow is subject, and advantage has already been taken of Herd B for this purpose. The effect upon the udder of other organisms, such as *Br. abortus*, could be determined with the assurance that the cows were free from streptococcus mastitis. (2) If a herd of this nature could be made available for experimental purposes, the spread of the various serological types into which Group I mastitis streptococci can be divided could be studied without possible confusion from the presence of specifically immune animals. (3) If, in addition, such herds could be freed from tuberculosis, abortion and Johne's disease, there could be obtained in course of time estimates of the milk yield

normal to the various breeds at different ages. In this way, standards could be prepared to which no objection could be raised. Other problems which could be investigated satisfactorily would be the factors which influence the milk yield of cows, and the comparative efficiency of hand and machine milking. (4) Qualitative and quantitative studies relating to the composition, reaction, cytology and bacteriology of normal udder milk could be undertaken in herds free from chronic streptococcus mastitis with greater assurance that the results would be acceptable. In our view, doubt must be cast on some of the results obtained in the past with udder milk which was accepted as normal merely because the cows appeared to be free from udder disease. This criticism applies most forcibly to the attempts which have been made to determine the cell count of cow's milk.

Summary.

Observations covering a five years' period were made in a practically self-contained herd consisting of 53 cows and heifers, of which, in June, 1928, 10 were affected with chronic streptococcus mastitis. Preventive measures, in which, during the first two years, the infected cows were milked last and subsequently kept on separate premises, have led to the formation of a group of cows which for three years and six months has remained free from the streptococci which cause the chronic and contagious form of mastitis. Of the 10 originally infected cows, two stayed on the farm for over four years, four for between two and three years and four for varying periods up to two years. Two other infected cows were located later. Altogether, five of those which were under observation for the longest time appeared to have made a complete recovery.

So far as can be judged from the literature, this is the first time it has been conclusively shown by frequent and searching tests of the milk that a herd of cows can be maintained free from infection. This constitutes the final proof that chronic streptococcus mastitis is a contagious disease and, therefore, theoretically preventable.

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OCULAR LESIONS IN EPIDEMIC BLINDNESS OF FOWLS.

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One of the symptoms which have been attributed to fowl paralysis is partial or complete blindness. The occurrence of blindness is, however, of somewhat variable incidence. In some outbreaks it has been rare, in others common, while in certain flocks blindness has been the only apparent symptom of ill health. The question has therefore been raised as to whether blindness in epidemic form can be regarded as pathognomonic of fowl paralysis in the absence of other characteristic symptoms of the disease.

Unfortunately the ocular lesions associated with epidemics of fowl paralysis have not been intensively studied. Marek (1907) in his original description of the disease failed to mention changes in the eyes, though Kaupp (1921), who first reported the condition in the United States of America, mentioned partial blindness as one of the symptoms, while Doyle (1926) noted the occurrence in fowl paralysis of a contracted non-responsive pupil which was regarded as being due to iritis. Pappenheimer, Dunn and Cone (1930) described in some detail the occurrence of ocular lesions in three birds suffering from partial or complete blindness in association with fowl paralysis.

In the living birds there was noted a change in the colour of the eyes from a yellow-brown to a bluish-grey. This was explained as being due to the lesions found in the iris, which was thickened, œdematous and infiltrated with wandering cells. These were of two distinct types, small deeply stained lymphoid cells with little cytoplasm, and large mononuclears resembling mammalian plasma cells with eccentric nuclei and basophilic cytoplasm. These larger elements were aggregated into compact clusters, situated more especially near the anterior surface. Occasional eosinophils were also seen. In the anterior chamber there was slight œdema, a granular coagulum, and in some sections a varying number of mononuclear cells of differing type. The cornea and retina were unaltered while the optic nerve heads showed only a minimal infiltration. Groups of lymphoid cells, however, were present in the conjunctiva and in the extraocular muscles. McGaughey and Downie (1930) also described the appearances in the eyes of two hens. In one the iris of the right eye showed an infiltration composed of eosinophilic and mononuclear cells. In the anterior chamber there was in addition an exudate composed mostly of eosinophils and polymorphonuclear leucocytes while in the cornea there was slight infiltration with the same types of cell. In the second hen the iris was infiltrated with cells resembling mammalian plasma cells. No changes were present in the optic nerve or retina.

These workers also found that iritis may be the only symptom present, while, since many of the infiltrating cells were of the eosinophilic and polymorphonuclear type, they agreed with the suggestion of Doyle (1926) that the ocular lesions may be due to some other agent than that responsible for the ætiology of ordinary fowl paralysis.

In view of these suggestions it seemed worth while to investigate rather more fully the lesions present in the eyes of fowls suffering from epidemic blindness with the object of throwing further light on the relationship between fowl paralysis and epidemic blindness in fowls.

Our colleagues, Miss Warrack and Major Dalling, therefore very kindly placed at our disposal the very large amount of histological material which they have collected during the past three years' work on fowl paralysis. This material comprises tissues obtained from birds experimentally infected in the laboratory, as well as from epidemics occurring on poultry farms in various parts of England. In certain of these epidemics partial or complete blindness was the only symptom noted, in others the blindness was associated with definite symptoms of fowl paralysis. This may be illustrated by the history of three, among many other, outbreaks from widely separated parts of Great Britain.

(I) Thirty pullets were purchased at three months of age ; two became lame within three days but were not sent for post-mortem examination. During the following nine months eight other birds became paralysed, while two others suffered from blindness and refused to eat. Examination of these birds showed a swelling on the left lumbo-sacral nerve plexus, a tumour beneath the skin on the left side of the sternum, both composed of cells of the lymphocyte series, and infiltration of the optic nerve.

(II). Fifty-two chicks of an R.I.R. strain were reared from day old chicks. There were no losses from coccidiosis, but after eight months one bird became lame and a month later was completely paralysed. At post-mortem examination there was a tumour mass involving the left sciatic nerve. At the same time blindness was noted in another bird which survived for nearly a year. At post-mortem examination the left lumbo-sacral nerve was swollen and the optic nerves were infiltrated by plasmacytoid cells. In addition an ovarian tumour composed of lymphocytes and plasma cells was also present.

(III). Among a flock of 150 chicks there occurred 18 cases of blindness. The blindness was confined to Wyandotte crosses with Rhode Island Reds and was not seen in stock mated with White Wyandottes from Ireland. No naked eye evidence of fowl paralysis was noted in this flock except in one hen in which there were large nodules of plasmacytoid cells and lymphocytes infiltrating the liver.*

Before discussing the evidence furnished by an examination of

* Within the last few weeks there have occurred in this flock typical cases of fowl paralysis, associated with tumours on the peripheral nerves.

this material on the relationship of fowl paralysis and epidemic blindness a brief account will be given of the ocular lesions encountered.

Pathological Changes.

Investigation of the tissues of birds exhibiting symptoms of blindness during life soon showed that in certain cases the cause of the blindness was to be found not in any lesion of the eye but in changes either of the optic nerves or in the optic tract of the brain itself. As is well known, in fowl paralysis perivascular infiltration may be present in the brain. The distribution of this perivascular infiltration in fowl paralysis is, however, very irregular, and in many

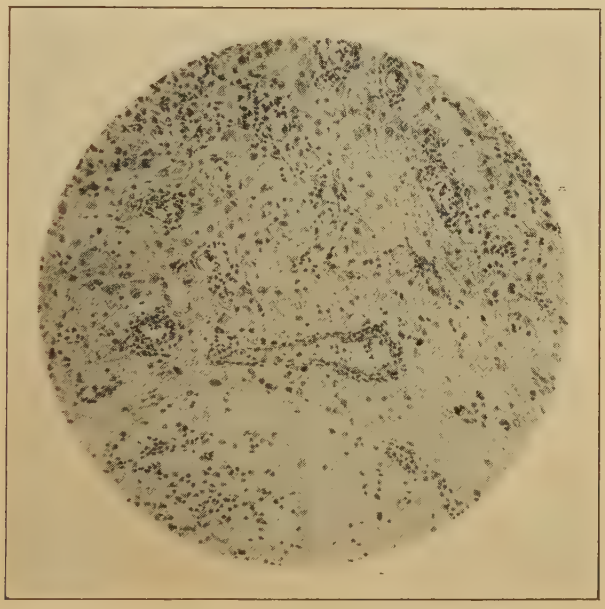

Fig. 1.—Occipital lobe of brain of hen with epidemic blindness : Perivascular infiltration. Mayer's haemalum and eosin. × 150.

brains it may be restricted to a small area. In certain cases it has been confined to the occipital lobes where, as in Fig. 1, the blood vessels are surrounded by a definite cuff of cells. Similar lesions have also been seen in the region of the thalamus. At other times the occipital lobes are the site of lymphomatous masses (Fig. 2) of which the cells, plasmacytoid cells and lymphocytes, are similar in type to those found in the perivascular infiltrations. The cells making up the perivascular infiltrations and nodular masses in the brains of birds suffering from blindness are thus similar in character to those encountered in the brains of birds suffering from typical fowl paralysis

but without any apparent involvement of vision. Plasmacytoid cells with typical clock-faced nuclei placed eccentrically in the cell are by far the most numerous type. Mitotic division is common among these cells. Very occasionally typical small lymphocytes are seen together with a few polymorphonuclear leucocytes. Unlike Pappenheimer, Dunn and Cone (1929), who reported perivascular infiltrations in the brain of supposedly normal chicks, we have not found this change present in this country in the brains of hens obtained from flocks with a negative history of fowl paralysis or epidemic blindness. In certain hens, the only apparent abnormality associated with blindness is a diffuse infiltration of the optic nerve. The infil-

FIG. 2.—Nodule, composed of plasma and plasmacytoid cells, in occipital lobe of hen with blindness. Mayer's haemalum and eosin. × 150.

trating cells are found in the thin connective tissue lamellæ which, as continuations of the pia mater, separate the nerve bundles. The infiltrating cells, which are again of the plasmacytoid type, with the addition of a very small number of polymorphonuclear leucocytes, are especially numerous round the small blood vessels which are present in the connective tissue septa. As a result of the extensive infiltration the nerve bundles are greatly disorganised.

The sclerotic coat was never found to be the site of extensive infiltrations, though such were common in the loose connective tissue in the orbit and also in the extrinsic muscles of the eyes, where in certain cases they caused wide separation of the muscle fibres. The

smaller accumulations of cells were always situated round the blood vessels and appeared to have originated from the walls of the perivascular lymph spaces.

Lesions of the choroidea were by no means rare. In the earlier stages the infiltrating cells were grouped round the medium-sized blood vessels of the vascular layer, but as the masses of cells increased in size they tended to involve the whole thickness of the choroid with resulting disorganisation of the flattened lamina suprachoroidea and the lamina vitrea.

Masses of pigmented cells, in part chromatophores of the choroid, in part pigmented epithelium from the retina, were thus in certain

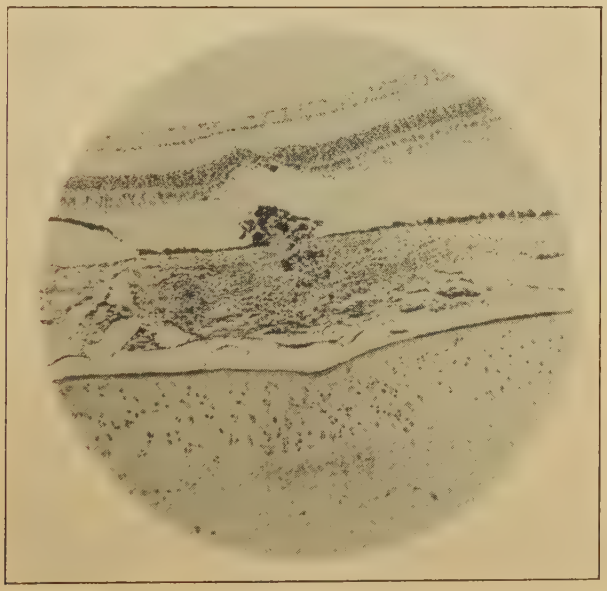

FIG. 3.—Infiltration of the choroidea with pressure on the rods and cones of the retina. Mayer's haemalum and eosin. × 30.

areas forced forwards on to the rod and cone cells, which at the point of pressure were flattened and destroyed while the nuclear layers were in their turn also thrust forwards into the inner reticular layer of the retina (Fig. 3). Here again the prevailing type of cell was of plasmacytoid type.

Apart from the effects of pressure due to the mass of infiltrating cells in the choroidea, the rod and cone cell layer of the retina was never found to be the site of primary accumulations of cells. The papilla of the optic nerve, however, was frequently infiltrated with dense masses of cells, while in the nerve fibre layer of the retina occasional accumulations of cells were by no means uncommon

(Fig. 4). As a result of these accumulations the internal limiting membrane was broken, while the ganglion cell layer was also disorganised. Various stages of degeneration were seen in the ganglion cells. Occasionally a few plasmacytoid cells were present in the vitreous. The pecten was also very frequently infiltrated with cells of a plasmacytoid character (Fig. 5).

The iris is probably the most common site of infiltration in the eye in cases of epidemic blindness in fowls. To this infiltration is due the change in size and colour of the pupil which is so frequently recognised during life as evidence of ocular involvement. As in the

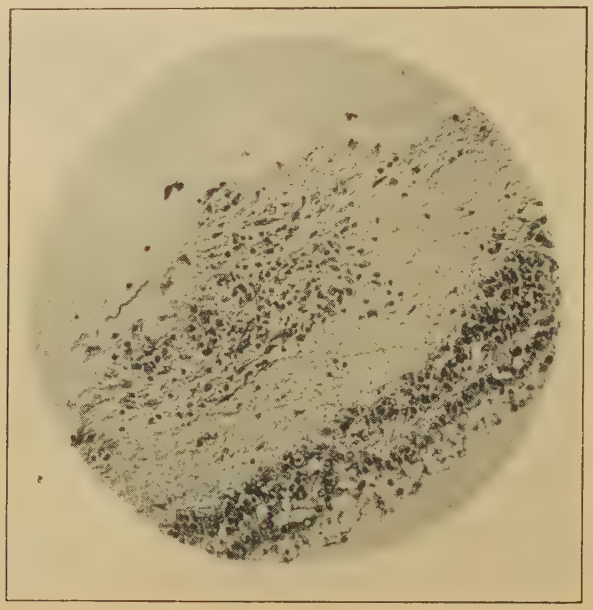

FIG. 4.—Infiltration of the nerve layer of the retina with destruction of the ganglion cells. Mayer's haemalum and eosin. × 200.

choroidea, the earlier lesions are found as perivascular infiltrations in the vascular layer, but rapidly involve the whole of the iris, which thus becomes thickened. The homogenous ground substance separating the melanophores, which under normal conditions contains fine collagenous fibrils not arranged in bundles, together with a few round cells and mast cells, is found to be packed with plasma-like cells and varying numbers of polymorphonuclear leucocytes. As a result of these dense masses of infiltrating cells the melanophores are isolated into small groups or single cells, the long branched processes of which no longer join together to form a loose cellular network. The epithelial pigment layer on the posterior surface of the iris as a rule remains intact, but occasionally the pigment cells are thrust

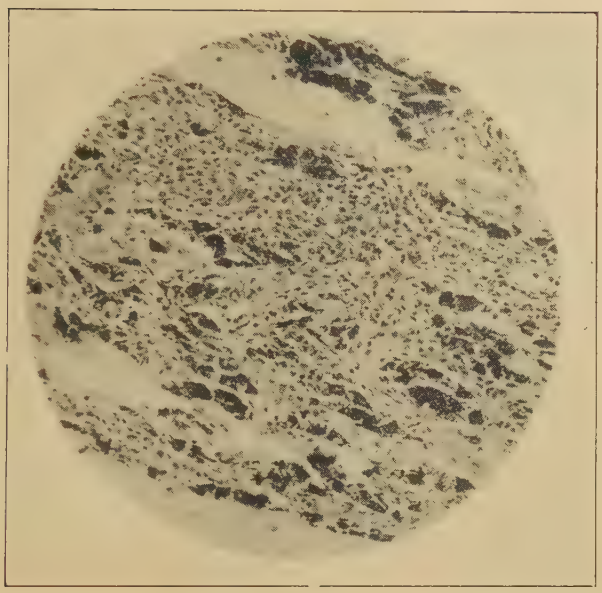

FIG. 5.—Infiltration of the pecten. Mayer's haemalum and eosin. × 150.

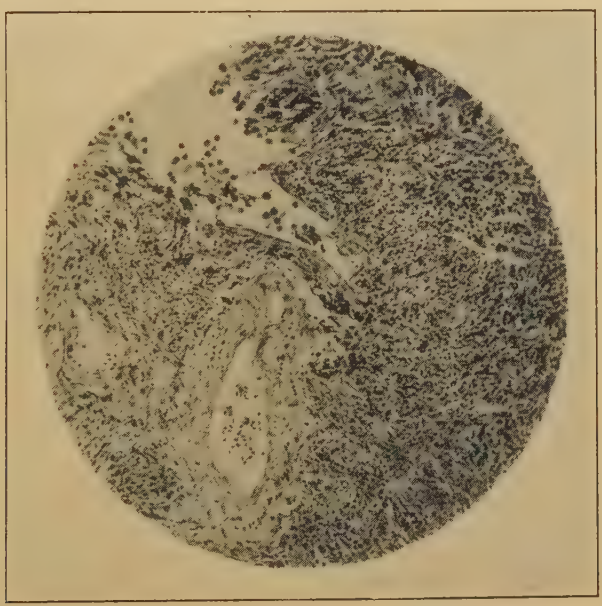

FIG. 6.—Infiltration of the angle of the iris. Mayer's haemalum and eosin. × 150.

aside by the infiltrating cells. The muscle fibres of the iris, on the other hand, are very frequently involved in the infiltrating process.

The angle of the iris, formed where the posterior surface of the cornea and the anterior surface of the iris meet to form a circular recess which in meridional sections of the eyeball presents itself as an angle, is very commonly the site of an extensive infiltration (Fig. 6), which may also involve the fibres of the ciliary muscle as well as the loose connective tissue surrounding the canal of Schlemm at the corneo-scleral junction. The cornea itself has only rarely been found to be invaded by plasmacytoid cells and then only when the tissues at the corneo-scleral junction or in the conjunctiva are the site of extensive infiltration. On the other hand, the anterior chamber of the eye not infrequently contains numerous cells of a plasma-like character, while masses of similar cells may also be found in the conjunctiva.

It will thus be seen that in fowls affected with epidemic blindness the impairment of vision may be associated with a variety of pathological lesions.

Types of Cell.

The types of infiltrating cell encountered in the ocular lesions of epidemic blindness have already been briefly mentioned. The plasma-like cells are of two types : (I) cells which correspond entirely with the mammalian plasma cell and are characterised, when stained with Giemsa's stain, by the presence of a rose-tinted demilune of cytoplasm in close proximity to the nucleus, and (II) cells without this rose-red area. These cells of the fowl, Mjassojedoff (1925) regards as atypical plasma cells. Certain of these plasma and plasmacytoid cells contain in their cytoplasm one or two small round basophilic bodies, 1μ to 3μ in diameter. With Giemsa's stain they take on a deep blue colour, though they are also well seen in sections stained with iron hæmatoxylin. These bodies cannot be looked upon as in any way specific, for very similar, if not identical, structures are seen in the cytoplasm of plasma cells in the lesions produced in man and in animals by the virus of lymphogranuloma inguinale. It is probable that the bodies represent either extruded nucleoli or phagocytosed cell debris.

In addition to the plasma and plasmacytoid cells, the lesions contain small numbers of small lymphocytes, a few large mononuclear cells, and occasionally fibroblastic cells, derived apparently from the fixed tissue cells. There are finally a very few basophilic mast cells and a variable number of eosinophilic polymorphonuclear leucocytes. Certain authors have described these cells as eosinophils. It must be remembered, however, that in the fowl there are no true neutrophil polymorphonuclear leucocytes, but cells having a polymorphonuclear-shaped nucleus and acicular eosinophilic bodies in the cytoplasm. True eosinophils corresponding to the eosinophils of mammals are very rare in the fowl. In certain ocular lesions eosinophilic polymor-

phonuclear leucocytes are present in considerable number, in other accumulations they are rare. This disparity in numbers may occur even in different lesions in the same bird. No definite virus inclusions have been detected in the cells, nor have bacteria been seen in the lesions.

The Relationship of Epidemic Blindness to Fowl Paralysis.

Since we have found no useful description of the histology of the eye in the normal fowl, we have examined the eyes of fowls and chickens from flocks in which there has never arisen any suspicion of the existence of fowl paralysis or epidemic blindness. The eyes of normal fowls do not exhibit infiltration with the cells characteristic of epidemic blindness. In one supposedly normal bird, however, from a flock which, so it was stated, had always been free from fowl paralysis and epidemic blindness, there was found a quite definite infiltration of the choroid with cells of mononuclear type. A few weeks after our examination had been made a number of birds in this supposedly healthy flock went sick with definite fowl paralysis, the clinical symptoms being confirmed by post-mortem examinations. There were, however, no clinical signs of eye involvement.

The characteristics of the cells found in epidemic blindness and their arrangement correspond so closely to those found in other organs in birds with a clear clinical history and macroscopic and microscopic evidence of fowl paralysis, that we are confident that histologically there is no clear differentiation between the lesions of epidemic blindness and those of ordinary fowl paralysis.

The suggestion that epidemic blindness in fowls is not due to the same exciting agent as fowl paralysis is based largely on the following evidence :—

(I) The number of fowls exhibiting signs of blindness varies greatly in different outbreaks of fowl paralysis.

(II) In certain epidemics blindness has been unaccompanied clinically by any other signs of fowl paralysis.

(III) The lesions in the eyes differ from the lesions of fowl paralysis in containing numbers of eosinophilic polymorphonuclear leucocytes.

The incidence of blindness, either partial or complete, varies in different outbreaks, but it must be remembered that, as shown in the present communication, involvement of the iris is not the only ocular lesion though it is the only one which is readily recognised clinically during life. Clinical evidence is, therefore, not a sufficient criterion for the exclusion of all ocular involvement.

Though in certain epidemics blindness may be the predominant or sole clinical symptom, nevertheless we are not yet convinced that in such outbreaks careful histological study of the brain, cord, or peripheral nerves will not yield evidence either of perivascular infiltration in the central nervous system or of accumulations of cells of the lymphocyte series in the peripheral nerves.

Finally, the presence or absence of polymorphonuclear leucocytes is hardly evidence of sufficient weight on which to base a difference in ætiology.

In certain diseases produced by filterable viruses, such as experimental poliomyelitis in monkeys and the encephalitis produced in the same species by the intracerebral inoculation of the virus of lymphogranuloma inguinale, the earlier lesions contain a considerable number of polymorphonuclear leucocytes which, as the disease progresses, are replaced by cells of the lymphocyte series.

In addition, in the infiltrations of the nerves, typical of true fowl paralysis, careful search always reveals the presence of a small number of polymorphonuclear leucocytes.

The evidence is therefore in favour of the view that epidemic blindness in fowls is one of the manifestations of true fowl paralysis or lymphomatosis, though, as emphasised by Bayon (1931, 1932), paralysis in fowls may be due to a number of causes. Till the actual cause of fowl paralysis is determined, however, it is impossible to be absolutely dogmatic on this point, since the possibility remains that two closely allied agents may be at work, one affecting the central and peripheral nervous system, the other attacking particularly the eye.

It may here be mentioned that this study has not in any way suggested that the affected hens are suffering from a deficiency of vitamin A, since corneal inflammation and degenerative changes in the para-ocular glands were conspicuous by their absence.

We desire to express our thanks to Miss G. H. Warrack and Mr. T. Dalling for their kindness in placing their histological material at our disposal and for much helpful assistance and advice.

Conclusions.

(I) Histological evidence is brought forward in support of the view that epidemic blindness in fowls is closely related to fowl paralysis (lymphomatosis gallinarum).

(II) Lesions associated with epidemic blindness in fowls were found in the optic tracts, optic nerves, choroid, retina, pecten, iris, intrinsic and extrinsic ocular muscles, as well as in the conjunctiva and connective tissue of the orbit.

(III) No evidence of ocular lesions due to lack of vitamin A was found in hens suffering from epidemic blindness.

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THE CONTROL OF RINDERPEST IN A LARGE DAIRY HERD IN SHANGHAI, CHINA.

By H. E. KEYLOCK, F.R.C.V.S.

WHEN a veterinary surgeon is called upon to arrest contagious disease amongst animals it is necessary for him to decide quickly upon a definite line of procedure, and in doing this he must take into account the results and conclusions reported by others. While the adopted line of procedure is being carried out, the results obtained may be found either to agree with the observations and conclusions of other workers or to differ from them in some respects. Further, as time elapses new results are reported, and these may make it necessary to modify one's previous conceptions regarding the disease and the methods by which it should be combated. In different parts of the world different conditions may make it appear as if the disease took on a different course, and suggest that results obtained in one country might not be obtainable in another.

The author's experiences with regard to the control of rinderpest from 1898 to 1909 were recorded in the *Journal of Comparative Pathology and Therapeutics*, Vol. XXII, 1909. Owing to his absence from China from 1910 to 1922 he was unable to make further observations regarding the disease, and the purpose of the present paper is to describe the results of renewed experience during the last ten years.

In 1910 the following views were held with regard to the control of rinderpest :—

1. The serum-simultaneous method (using bovine virulent blood concurrently with anti-serum) conferred a permanent immunity against rinderpest, but the method involved considerable risks, (*a*) because of unforeseen variations in the virulency of the blood, (*b*) because the virulent blood might contain piroplasms, or (*c*) the reaction to the inoculation might resuscitate a dormant piroplasmosis in the inoculated animal.

2. The anti-rinderpest serum employed varied but little in potency if it was derived from the mixed bleedings of a number of animals, and its potency was retained for at least two or three years.

3. Anti-rinderpest serum could be used alone in an endeavour to arrest and control an outbreak of the disease, and, provided the temperatures of all in-contact animals were taken daily, an accurate forecast of deaths and recoveries could be made.

4. The disadvantages of the serum-alone method were (*a*) that no dose of serum could be relied upon to ward off the disease for a longer period than eight days ; (*b*) that, since animals possessed varying degrees of resistance to contact infection, a very considerable period had to elapse before a herd could be considered free from further casualties, and to obtain the most successful results daily visits and inoculation had to be made.

Such were the views we still held in 1922, when it became necessary to search again for a satisfactory method of preventing infection, for, having the care of a large dairy herd, the purpose of which is to provide a continuous supply of milk, an outbreak of rinderpest would very seriously affect the output of milk.

Further Experiences with the Serum-Simultaneous Method.

Although others had described and recommended the simultaneous method of immunisation, it was felt that, in view of our previous local experience, such a method would be extremely dangerous, and that the most that could be attempted was to give the method a further trial upon animals up to 12 months old, bred in the dairy.

Inoculations of the young stock in this way were undertaken, but as losses were again excessive, many animals exhibiting symptoms of piroplasmosis, the simultaneous method was once more abandoned. Until 1927 only bovine virulent blood had been used in the inoculation. In January, 1926, rinderpest broke out in the dairy and anti-rinderpest serum was freely used, with results shown in the following table.

Table A.

January, 1926.

Animals.	*Number.*	*Affected.*	*Died.*	*Re-covered.*	*Not Affected.*
Not involved in previous outbreak ...	112	56	6	50	56
Involved in a previous outbreak ...	39	—	—	—	39
Involved in two previous outbreaks ...	24	—	—	—	24
Had previously been inoculated with serum and virulent blood	62	—	—	—	62
	237	56	6	50	181

The conclusions drawn from this outbreak were :—

1. Large doses of serum (300 c.c. or more) must be given to animals in advanced pregnancy to confer adequate protection.

2. In an outbreak animals that receive serum acquire immunity although they show no clinical symptoms (not even a slight temperature).

3. An outbreak of rinderpest should occasion very little loss if there is prompt application of serum to all susceptible animals. The longer the delay in the application of the serum the greater will be the losses.

Whether those animals that had not exhibited noteworthy or prolonged symptoms had derived solid lasting immunity or not was an interesting question that remained to be solved.

The Use of Goat, Instead of Bovine, Virulent Blood in the Serum-Simultaneous Method.

In 1927 it was noted that Hutyra and Marek claimed that goats were not susceptible to piroplasmosis, and, as it was found that these animals could be infected with rinderpest, virulent rinderpest blood from the goat was used to inoculate young cattle by the simultaneous method.

It was found that the losses occurring in young stock from serum and goat virus were inconsiderable, and all the young animals were thus treated.

The goat virus was obtained by inoculating, as a routine measure, virulent bovine blood into three goats. About 70 per cent. of the goats were found to give a temperature reaction within 96 hours. A goat showing a good reaction was selected, blood was taken from the jugular or carotid, and 1 c.c. of the blood was injected as a dose in the serum-simultaneous inoculation. The virulence of the blood was always confirmed by inoculation into three bovines, which were usually employed afterwards for serum production. No attempt was made to passage the virus in goats ; hence our work does not resemble that which has recently been reported from India upon attempts to immunise cattle by inoculating them with attenuated virus in the form of a goat adapted virus. Our work was directed primarily to overcoming the risk of accident due to concomitant piroplasmosis, and this we were led to believe could be achieved by inoculating the cattle to be immunised with virulent rinderpest blood uncontaminated with bovine piroplasmosis from a donor, namely the goat, which was not susceptible to infection with these piroplasms.

History and Control of Outbreak B.

From July, 1927 to January, 1929, rinderpest was very prevalent in the Shanghai district, but the dairy escaped infection although suitable dairy cattle were being freely accepted from local Chinese farmers. Also during this period shipments of highly susceptible cattle were received from England and Canada. We had decided that such isolation methods as we could enforce in Shanghai would be ineffective, and that the only reliable method of control was by prophylactic or curative treatment.

In January, 1929, rinderpest again attacked the dairy, the disease originating in a locally purchased animal. The state of the animals as regards their susceptibility, the treatment applied, and the results obtained are given in the following table.

It will be seen from the table :—

(*a*) *Animals that had passed through a previous outbreak.*—Among the 89 animals there were 30 Friesian cattle which had arrived from Canada in October, 1925, and all were in the dairy when rinderpest broke out in January, 1926 (Outbreak A). In this outbreak 28 of these Friesians (the other two are referred to later) received large

TABLE B.

OUTBREAK B.—JANUARY, 1929.

	Animals believed to be Immune.		*Animals believed to be Susceptible.*				
	No Treatment.		*Treatment.*				
				Received Serum only.		*Received Serum and v.b.*	
	No.	*Deaths.*	*No.*	*No.*	*Deaths.*	*No.*	*Deaths.*
Animals that had passed through a previous outbreak	89	—	—	—	—	—	—
Animals that had received the simultaneous inoculation :—							
Adults	86	1	—	—	—	—	—
Young stock (6 to 30 months)	58	—	—	—	—	—	—
Calves up to 6 months	71	—	—	—	—	—	—
First Jersey shipment (Canada...	—	—	32	8	5	24	—
First Shorthorn shipment (England)	—	—	7	3	1	4	—
First Harbin shipment (Manchuria	20	5	—	9	5	11	—
Second Jersey shipment (Canada)	—	—	10	—	—	10	—
Second Friesian shipment (Canada)	—	—	10	3	1	7	—
First Japanese Friesians shipment (Japan) ...	—	—	7	6	3	2	1
Locally obtained animals (Shanghai)	5	1	82	11	2	69	2
	329	7	148	40	17	127	3

doses of serum, and subsequently five exhibited symptoms of the disease, while the remaining 23 showed no symptoms—not even a definite temperature reaction. During Outbreak B (January, 1929), of the above Friesians there were 20 still on hand on the premises, and five of them had exhibited symptoms in Outbreak A, while 13 had not shown symptoms. During Outbreak B none of these 18 exhibited a reaction. The 13 that had shown no reaction in either Outbreak A or B, were given virulent blood and serum again, and again there was no reaction. The two animals omitted from the above count (Nos. 522 and 525) were in an advanced stage of pregnancy at the time of Outbreak A, and they had received large doses of serum and were isolated. They did not contract the disease. During Outbreak B they were given virulent goat blood and serum and then showed no reaction. It was concluded that these results had answered the question which arose after Outbreak A regarding the degree and duration of the immunity acquired by an animal passing through an outbreak without showing any clinical symptoms, and showed that such an acquired immunity was effective for a period of at least three years.

(*b*) *Animals that had received the simultaneous injections.*—The 86 adult animals and the 129 head of young stock from 6 to 30 months old had all received virulent goat blood and serum, the inoculations being made when the animals were about 6 months old and repeated at some convenient time when they were from 12 to 36 months old.

Among both the adults and the young stock only two showed serious symptoms during the outbreak, and one of these died. Many of the animals gave temperature reactions. From the results it was concluded that the simultaneous injections had not conferred a solid immunity upon some animals, and upon others no immunity at all, or at least, not an immunity that was sufficient to prevent a fatal issue at the time of the outbreak, but that the loss from deaths must be regarded as inconsiderable.

It may be mentioned that the temperature of every animal in the dairy is taken and recorded daily, and this procedure is a routine practised every day in the year.

(*c*) *The Harbin shipment.*—The animals of this lot were stated to have been immunised against rinderpest in Harbin before shipment, but they proved to be non-immune. As they were believed to be immune, the serum injections were not given as early as they should have been.

(*d*) *Animals known to be susceptible.*—Of these we will consider 59, namely, the first and second Jersey shipments, the second Friesian shipment, and the shorthorn shipment, as there can be no doubt as to the susceptibility of these batches, and they were animals maintained under similar conditions to those of future importations.

Seven of these animals were sent to the Infirmary where the serum was prepared, and where they were at once given a dose of virulent goat blood and serum. They gave temperature reactions

only, and no other symptoms whatsoever. They were exposed to infection during the whole of the time at the Infirmary.

Of the remaining 52 all received a dose of serum at the commencement of the outbreak ; two developed fever within 24 hours, and one of them died ; 32 developed fever at varying intervals extending to three days after the serum injection, and five of these died.

Animals that gave no temperature or other evidence of the disease received virulent goat blood and serum. None died as the result of the injection with virulent blood. As two gave no reaction to the injection of virulent blood and serum they were given a second dose of virulent blood, and as one still gave no reaction a further dose of virulent blood was given again without effect.

So far as the losses are concerned the injections of virulent blood cannot be held to have had any bearing upon them, and the fatalities would undoubtedly have been the same had virulent blood not been used. But, would the losses have been as heavy, or heavier, if virulent blood had been used with the first dose of serum ?

An injection of serum every four days will prevent natural infection indefinitely, but if, in the face of an outbreak, serum is not given on the fourth day following upon the last injection, infection occurs and a temperature reaction is evident about the ninth day. The majority of these animals will recover if they are given a second dose of serum directly their temperature rises.

It is not practicable to give serum to all susceptible animals every four days, owing to the large quantity of serum that would be required ; it also cannot be known when it would be safe to discontinue the injections. Therefore, one of two procedures may be adopted, namely, either to give a dose of serum and allow the animal to contract the disease from contact infection, or give a dose of serum and virulent blood to all susceptible animals as soon as the disease makes its appearance.

The advantages and disadvantages of these two methods were not compared, because at the time we had none but immune cattle (of all ages) on hand and the obvious procedure was to immunise all animals bred at the dairy and all future importations as they arrived at the dairy.

During the outbreak under review our losses were far heavier than we had anticipated and were due to the following reasons :—

1. The Jersey breed of cattle appear to be more susceptible to the disease than any other.

2. We cannot control the disease in an animal that is well advanced in pregnancy, say over six months. We can, with large doses of serum, keep the animal free from the disease until it has calved, but after that event, in the majority of the cases, nothing appears capable of preventing a fatal issue.

The first statement explains most of our losses in the Jersey shipment, and the second statement explains our losses in the Japanese

shipment, as these cattle arrived a few days before the disease made its appearance and all were due to calve within a month.

It will be observed that our percentage of losses in the 1929 outbreak was approximately double that of the 1926 outbreak ; and the circumstances make it difficult to state precisely the reason, but the explanation may be that the Jersey breed is more susceptible than any other breed, and the arrival of the eight Japanese cows at the moment of the outbreak was extremely unfortunate. On the other hand, the smaller percentages of losses during the 1926 outbreak may have been due to some immunity having been conferred by our previous periodical injections of glycerinated bile. Parenthetically it may be mentioned, with reference to bile, that we have arrived at the conclusion that it may or may not have immunising properties, but there are no physical or other known characteristics to indicate whether a given bile possesses such properties. We think that, until some method of ascertaining the potency of bile is discovered, the injection of bile may lead to a false sense of security, and a more certain method of conferring immunity is desirable. In addition, it may be noted that bile is very expensive to produce.

The control of a rapidly infectious and very fatal disease such as rinderpest is far more difficult in a large dairy than in a small one, and we have not the benefit of the experience of others to assist us, for, as far as we know, such data have not been recorded; consequently, unforeseen problems constantly arise. As the lowest effective dose of serum is 100 c.c., and we sometimes find it necessary to give as much as 700 c.c. as a dose, and these doses may have to be repeated two or three times in the course of an outbreak, it will be readily understood that very large quantities of serum must be available, and the larger the dairy the more acute this question becomes.

At the commencement of the outbreak we had 241 animals that needed serum and 233 that were immune. As we cannot allow less than 500 c.c. of serum per animal, we needed at least 120,000 c.c. of serum immediately (it takes at least three months to make it), which quantity we were fortunate enough to have on hand. But as serum does not retain its potency indefinitely, it would not be practicable to carry in stock a very much greater quantity, apart from the fact that it is expensive to make and represents a considerable sum in locked-up capital.

Even if an unlimited supply of serum were available there is another serious side to this question, namely, that since an animal's milk supply is arrested when the disease is contracted our milk output is at once affected.

We are therefore faced with the following two facts :—

1. The quantity of serum likely to be available at the inception of an outbreak is limited, and the number of non-immune cows ought therefore to be as low as possible.

2. The output of milk is liable to be depleted at any moment by an outbreak as long as we have non-immune cows in the dairy,

and the extent of this possible depletion will depend upon the number of non-immune cows.

In view of the above two facts we cannot secure a continually increasing milk supply under our previous method of combating rinderpest, namely, by the immunisation of young stock and treatment of imported animals with serum when attacked. Our only satisfactory solution of the problem is to immunise all imported animals as soon as we can after their arrival.

We have solid grounds for estimating that our losses from the immunisation will not exceed 6 per cent., but even if they amount to double that figure it will be an expense collateral to the maintenance of a continuous milk supply.

From February, 1929 to April, 1932 the procedure adopted was as follows :—all imported animals which were in-calf heifers received upon arrival a large injection of serum and, after calving, they were given virulent goat's blood and serum. All stock born at the dairy received virulent goat blood and serum at about six months of age, and these injections were repeated in from 12 to 18 months.

The reactions were very irregular notwithstanding the dose of serum given, but at this period we were under the impression that a sufficient immunity would be conferred by the simultaneous method, even if no clinical reactions were exhibited after the inoculations. This view is now believed to have been erroneous ; a decided temperature reaction is necessary to confer immunity. Virulent goat blood was always used, but on at least two occasions symptoms of piroplasmosis followed the injections. During the past four years we have arrived at the conclusion that, in a country where piroplasmosis is present, there are no means by which one can obtain certainty that bovine blood is free from piroplasms, and that, as concurrent piroplasmosis (of any variety) and rinderpest, even when the attack is not fatal, will leave an animal in a very debilitated condition, bovine blood must not be used for simultaneous inoculation.

It has been stated that goats are not susceptible to piroplasmosis, but this statement does not exclude the possibility of their acting as " carriers." It may be noted that J. G. Thomson and A. Robertson, in their book *Protozoology*, 1929, make the following statement :— " There are three species of Babesia found in sheep and goats, *viz.*, *B. sergenti* (Wenyon, 1926), *B. motasi* (Wenyon, 1926), and *B. ovis* (Starcovici, 1893). The *B. motasi* resembles the *B. bigemina* in size. The three species which are found in sheep and goats are in many respects similar to those found in cattle." Piroplasmosis in cattle has on two occasions followed simultaneous injections of serum and virulent goat blood, but we are not prepared to say that the occurrence was not attributable to the resuscitation of a dormant piroplasmosis.

In the preventive treatment of rinderpest by the serum-alone method it is quite obvious that one breed of cattle is more resistant to the disease than another breed and that one animal of the same breed is more resistant than another of the same breed ; and, since

a reaction is necessary to secure immunity, the exact dose of serum cannot be accurately calculated. Therefore, to obtain a reaction in some cases repeated visits and inoculations with regular accurate temperature recordings are necessary. Such a procedure is practicable only under very exceptional conditions.

The virulence of rinderpest blood is easily and rapidly destroyed, and certain observers state that for the simultaneous method it must be used within four hours of withdrawal and protected from light. We have found that virulent blood, collected and stored in bottles that have been sterilised by dry heat, will convey the disease after 48 hours, even if it has been exposed to the light in an ordinary room ; but, as the virulence is easily destroyed by disinfectants, great care must be taken not to soil the hypodermic syringe and needles with these agents. One drop of virulent blood will convey the disease as rapidly and surely as 20 c.c. or any larger dose, and, in our experience, when the disease is conveyed by the injection of virulent blood a rise of temperature always occurs within 72 hours. If the temperature does not rise over three degrees within this period but rises after 72 hours have elapsed, then the disease has not been conveyed with the virulent blood, and the rise of temperature is due to natural infection or perhaps the onset of piroplasmosis. We cannot accept as a proved occurrence what is termed a " delayed reaction."

We have never been able to trace the source of rinderpest except on the view that the disease can be conveyed by food-stuffs, but, even so, it can hardly be a coincidence that an outbreak should recur so regularly in the herd every three or four years. It seems highly probable that Daubney has supplied the explanation (Kenya Department of Agriculture Annual Report, Year 1929, p. 216), when he says that the immunity to rinderpest conferred by simultaneous injections gradually decreases, and that when an animal is exposed to infection before the immunity has vanished it will contract a symptomless infection.

We have not the slightest doubt that these observations are correct, and that it is the explanation of what has occurred in this dairy. Previous to 1929 the disease attacked the dairy every three or four years, and after an attack all the remaining animals had acquired immunity. As new animals were brought into the dairy so the chances of infection by food-stuffs increased, until one or two contracted the disease and infected the remainder of the susceptible animals, and infection by contact again raised the resistance of those animals that had survived the previous outbreak. Since the last outbreak in 1929 we have had isolated cases ranging in severity from a fatal issue to a slight temperature, and no doubt these cases were reinfected by the animals that were undergoing immunisation by the serum-simultaneous method. The number of deaths in our experience with this method has not been heavy, but the reactions have been unaccountably severe, leaving the animals in such a debilitated condition that the indirect loss has been excessive.

Conclusions.

The serum-simultaneous method of conferring immunity against rinderpest entails considerable risks, produces an uncertain degree of immunity, and causes severe economic loss. It therefore ought to be avoided if other methods of protecting cattle against rinderpest are possible.

Daubney's reports from Kenya indicate that formalised splenic vaccine will confer immunity that will last for at least 12 months, and we have employed that method since April, 1932.

It is believed that specific bovine piroplasmosis may be carried by virulent rinderpest goat blood when it is used as an inoculum in the simultaneous method of protecting cattle against rinderpest.

THE PREPARATION OF JOHNIN FROM A SYNTHETIC MEDIUM WITHOUT THE ADDITION OF *B. PHLEI.*

By G. W. Dunkin.

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Up to the present time it has been possible to cultivate the bacillus of Johne's disease only with the addition of *B. phlei*, certain extracts of this bacillus, or some other acid-fast organism to the medium. Twort and Ingram (1913) were the first to ascertain this important fact and it has been accepted ever since. Sometimes these authors used *B. tuberculosis* and at other times *B. phlei*, and they found that the " essential substance " was present in both.

Since that time acid-fast organisms have invariably been a component of all media on which it was intended to cultivate the bacillus of Johne's disease. The other constituents have varied according to the fancy of the worker. Even with the presence of the " essential substance," whether derived from one or other member of the acid-fast group, one could not with certainty prophesy a luxuriant growth, and in the author's experience it varies considerably from time to time, even with the same strain of Johne's bacillus.

In 1928 the author recorded the results of his experiments designed in the first instance to secure a good uniform and, if possible, luxuriant growth on the surface of a liquid medium with a view to preparing a potent johnin. Prior to that time some twenty or more strains of organisms had been obtained from actual cases of the disease occurring in different parts of the country. Primary cultures were first obtained on the surface of the solid medium described (1928), and subsequently sub-cultured when necessary. It was observed that while some of these strains were exceedingly slow in their rate of growth others grew with remarkable rapidity, while still others were intermediate in growth rate. This characteristic persisted even with the fourth and fifth generations, and it was therefore decided to mix three strains together in approximately equal amounts ; for this purpose a quick, a slow, and a medium growing variety were used, and these strains have been called " Stafford," " Peto " and " C.28 " respectively.

Although every effort was made to ensure that exact amounts of these strains of organisms were mixed, actual weights were not taken. Nevertheless, the rate of growth of the mixture on solid medium was as rapid as that of any of the individual strains, control cultures of which were grown side by side in the same incubator. This phenomenon was noted also when growth took place on the surface of the liquid medium, and it has been continued to the present day.

The johnin prepared from such cultures on liquid media has now

been in constant use in this country and some 256 veterinary surgeons have reported their results. The double intradermal method of testing has been universally employed. There is much evidence that the johnin described has played a useful part in the detection of the disease in its early stages and in a few instances has allowed the owner to commence eradication measures.

The occurrence of a non-specific swelling at the site of inoculation is a drawback to the use of the product in its present form. This has attained at times such dimensions and characters that it has been confused by the inexperienced with a true reaction. It became evident that efforts must be made to eliminate the cause of this non-specific swelling, and work designed to bring this about has been in progress for the past three years.

One of the results of these investigations is the establishment of the fact that certain bovines which react to both johnin and tuberculin, to either of these or to neither, may give a positive reaction to a phlein prepared on lines similar to those adopted for the preparation of tuberculin.

The concentrated broth employed by the author (1928), even without *B. phlei*, will produce a swelling which might be confused with a true reaction when injected intradermally into some animals.

Skin sensitivity of tuberculous patients to non-specific protein substances has, of course, been observed by many workers. D'Arcy Hart (1933) obtained qualitatively similar skin reactions to the characteristic delayed response to tuberculin in a large number of individuals by the intracutaneous injection of glycerinated veal peptone broth. He also found that a larger quantity was necessary to produce the reaction than of tuberculin.

A further conclusion arrived at by the same author is that the incidence of skin sensitivity to glycerinated veal broth is practically identical with the incidence of tuberculin hypersensitiveness. Tytler (1930), in attempting to explain non-specific reactions in general, suggests that the effect is quite independent of tuberculin sensitivity and that any apparent parallelism is accidental ; alternatively, that in tuberculosis there is an increased sensitivity to irritants in general, and that the development of sensitivity to a specific bacterial protein involves some increased sensitivity to bacterial proteins in general or an increased capacity to develop such sensitivity.

Whereas the experiments of D'Arcy Hart and the suggested explanations of Tytler all refer to tuberculosis in humans, it is not unlikely that a parallel explanation may be accepted in the case of Johne's disease of cattle.

It was quite clear, therefore, that for success to be attained the medium itself would have to be less complex and, if possible, the addition of dead acid-fast organisms should be omitted.

W. A. Hagan, Cornell University, in a private communication, informed me of his success in cultivating the organism of Johne's disease on the surface of a synthetic medium described by Henley

(1929) and since 1931 this medium has been in constant use. Its composition is as follows :

K_2HPO_4	.. ..	1·0 gm.
Na. cit.	.. ..	0·5 gm.
$MgSO_4$	.. ..	1·0 gm.
Asparagin	.. ..	5·0 gms.
Ferri cit.	.. ..	0·06 gm.
Glycerin	.. ..	70 c.c.
Dried *B. phlei*	.. ..	0·3 to 0·5 gm.
Aqua distil.	.. ..	1,000 c.c.

This medium differs from that of Long (1926) in that there are no ammonia salts present, that the percentage of glycerol is greater, and finally that it contains 0·3 to 0·5 gm. of dried *B. phlei* per litre, which is also absent from Henley's original medium.

Several attempts were made to cultivate Johne's bacillus on the surface of this medium but only meagre growth was observed. When the culture medium had been in the incubator for three months and no macroscopic evidence of growth was seen it was discarded. Sub-cultures were made from those flasks in which growth, however slight, was apparent. This procedure was continued for five generations and it was not until the sixth generation was reached that the growth could be described as good. On this occasion twelve flasks, each containing 250 c.c. of medium, were implanted, and only six of these contained the dead *B. phlei*. Each of the twelve flasks was inoculated at the same time and from the same growth, and they were subsequently transferred to the hot room where they were kept at a temperature of 39° C.

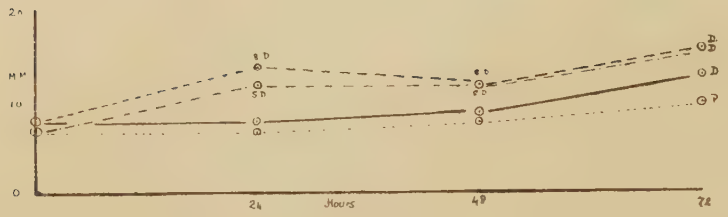
At first it appeared that the result was to be the same as that already noted in previous attempts, but by the end of two and a half months growth was apparent in the flasks containing *B. phlei* and by three months it was seen to be continuing. As yet, however, no evidence of multiplication could be detected in the flasks without *B. phlei*, and it was not until the lapse of three and a half months that definite signs of growth in these flasks was seen. In the author's experience this was the first time that this had occurred. In both series of flasks growth was exceedingly slow, and it was not until the expiration of four and a half months that the surface of the medium in the majority of the *B. phlei* flasks was completely covered with growth. At this stage none of the other flasks had advanced to that extent, and on the best of them the growth was not quite half the area of the fluid surface ; in the others the area covered was less extensive.

It was, however, decided to harvest the growth and to keep the products from the two sets separate from each other. The organisms having been filtered off through paper, the filtrate was reduced to one-tenth its original volume on a water bath. The resultant product from the phlein medium was of a deep amber colour, whereas that of the medium without *B. phlei* was of a lighter tint. The colour of

S = Slightly
D = Diffuse
T = Tender
B = Bean-like
P = Pea-like

Ordinary Johnin Batch 27 ——— ——— ———
Synthetic Johnin with B. PHLEI ——— · ——— · ———
Synthetic Johnin NO B. PHLEI ———
Conc Synthetic Medium. · · · · · ·

C 94



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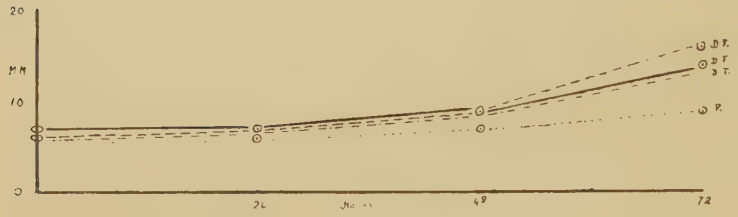

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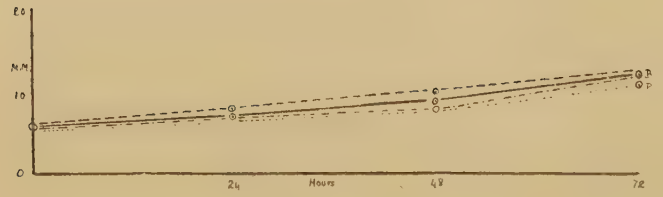
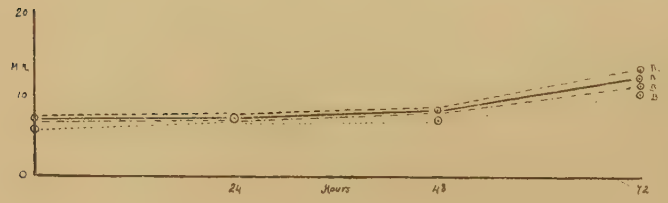
Ordinary Johnin Batch 27 ——— ——— ———
Synthetic Johnin with B. PHLEI. ——— · ——— · ———
Synthetic Johnin NO B. PHLEI ———
Conc Synthetic Medium · · · · · ·

C 66





the concentrated medium on which no organism had been grown and in which *B. phlei* had not been suspended was of a dirty white colour, the difference in appearance between this material and the johnin without phlein indicating quite clearly that a product or products of the bacillus of Johne's disease was present in the fluid.

The next step taken was to test these products on bovines, and for this purpose two animals known to be reactors to ordinary johnin but not to tuberculin (C.99 and C.122), and two other bovines which gave negative results to both johnin and tuberculin (C.66 and C.91) were selected. Each animal received on one side of the neck 0·2 c.c. of the products described above and also of ordinary johnin. The dose was repeated at the forty-eighth hour after the usual measurements and observations on the swelling had been made, and a diagnosis was reached 24 hours later.

The attached charts indicate the result of these tests, and it will be seen that in the case of reacting animals positive results were obtained with all three johnins, whereas the control medium produced a slight pea-like swelling which it was impossible to confuse with a true reaction. It will also be seen that the phlei-containing johnin gave in one instance a larger swelling than was obtained with the phlei without johnin and in the remaining reacting animal they were of the same magnitude. In each case, however, there was no doubt about there being a reaction, for, whilst in the one instance (C.99) the increase in swelling noted which resulted from the injection of johnin without phlei was only 5 m.m., it was clearly diffuse although not recognisably tender. In the other case it was both diffuse and tender.

The results observed in the normal beasts were negative throughout.

Discussion.—Considering the paucity of growth observed in the cultivation of the bacillus of Johne's disease grown on media not containing *B. phlei*, the potency of the resulting johnin is definitely encouraging. Nevertheless the degree of potency should be capable of considerable improvement, and it is thought that this can be achieved without running the risk of increasing the non-specific swelling; for instance, it is probable that further concentration might achieve this result, but owing to the small amount of the final product available this has not been tried. Further tests in the field are contemplated as soon as suitable arrangements can be made.

Summary.

On one occasion successful cultivation of the bacillus of Johne's disease, grown on the surface of a synthetic medium (without the addition of acid-fast organisms or their extracts), has been obtained.

Johnin, although not of high potency but possessing allergic activity as shown by tests applied on four selected bovines, has been prepared.

These two factors coupled with the complete absence of non-specific swelling suggests a fruitful line for future investigation.

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AN OUTBREAK OF SURRA AMONG THE WILD DEER (*CERVUS UNICOLOR VAR*) OF MAURITIUS.

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The disease "Surra" was brought into Mauritius in domestic cattle imported to the island from India in 1901. It rapidly spread in acute epidemic form among the local bovine and equine populations and thousands of animals succumbed to it in a few months; this caused a serious economic crisis, as the tending and harvesting of the sugar crop, the island's staple industry, turned on animal transport. Diagnosis of the condition was not made for over six months and when, finally, the trypanosome was discovered and treatment instituted the mortality in the surviving bovines rapidly decreased. Since the few years after its introduction surra has been present in endemic form, and sporadic cases are reported irregularly from all parts of the island. Equines, by law, are destroyed as soon as a diagnosis of surra is made in them, while bovines are segregated and treated with intravenous atoxyl and arsenious acid by the mouth; they respond well to this treatment.

Twenty-two cases in all were actually reported to the Agricultural Department from various parts of the island in the first six months of 1933, five of which were in equines and the remainder in cattle.

A few deer (*Cervus unicolor var*) were introduced into Mauritius from Batavia by the Dutch in the year 1639. These animals were turned down and have multiplied, and, in default of any natural enemies, have over-run the island. They are found largely in the forested areas, particularly on the higher ground (500 to 2,000 feet), and feed mainly in clearings planted with grasses introduced for their benefit. The cervine population now numbers about 20,000 head, according to a recent rough census, and the animals are legally protected during a long close season and are strictly preserved by various landed proprietors and sporting gentry. The concentration of animals will therefore be seen to be considerable when it is borne in mind that the total area of the island is only about that of the county of Surrey. In 1884 an epidemic, diagnosed as rinderpest, appeared among them and a large number died. Since that time there is no record of any abnormal mortality among them, and it is incredible that such could have occurred without the knowledge of the watchful estate owners and their numerous keepers. Rinderpest, it may be added, is now nknown in the country. In April and May of this year it was noticed

that a large number of deer were sick and dying on an estate, Solesse, in the south-east portion of the centre of the island. The owner and his keepers stated that to their knowledge over 60 beasts had perished by the middle of May and that many more were sick. The symptoms were apparently those of acute fever and rapid loss of condition. We were requested to conduct an investigation into the cause of the mortality.

A visit was made to the forest towards the end of May and the ground was found to be heavily stocked with animals, the available grazing area being eaten almost bare. About 120 deer were observed in an open " plaine " of approximately 60 acres ; these were all females and fawns with the exception of two or three young immature males. The animals of this forest apparently resort daily in the afternoons from the woods to this spot to feed. Three or four of those under observation were obviously acutely ill, and were lying about while the others were feeding. An old doe from among the former was stalked and shot. On post-mortem examination she was found to be in exceedingly poor condition and very anæmic ; the coat was rough and staring and the animal had obviously recently lost a considerable amount of flesh. On opening the abdomen a considerable amount of fluid was found in the peritoneum and pericardium. The lymphatic glands were but slightly enlarged ; no œdematous areas were to be seen round the abdomen, the genitalia, or the limbs, and nothing else abnormal was observed. Smears and swabs were taken from a number of organs for direct examination and culture ; on study of stained films from the heart's blood the presence of large numbers of a monomorphic trypanosome, indistinguishable from *T. evansi*, was revealed. Three further sick does were destroyed and similarly examined in the following few days and the post-mortem findings were alike in each case ; in each of them the heart blood contained large numbers of a similar trypanosome. No other pathogenic bacteria or protozoa were found, and the intestines were singularly free from helminths. One of these sick beasts was too weak to rise and attempt to escape and was readily caught by hand.

The hunting season opened at the beginning of June and a further visit was made to the forest about the middle of the month. During an extensive drive, only but a fraction of the usual number of deer, both stags and does, was encountered ; stags only were shot, ten being killed where in former years from 40 to 60 were obtained. The heart blood of each of these ten animals was examined, but no parasites were found in any of them ; the beasts were all in good condition and showed no signs of illness. During the drive the beaters found the carcases of 16 dead animals and, on enquiry, it was found that this brought the total of animals known to have died to date up to about 160. An interesting statement was made by the keepers to the effect that a number of pariah dogs which had fed on carcases of dead deer had themselves died a few weeks later. No further sick beasts were encountered during this drive, but the owner informed us that

there had recently been a recrudescence of the disease in the herd. Many *Stomoxys nigra* were seen feeding on the bodies of the dead animals between four and five hours after they had been shot.

It is of interest to note that this is the first occasion on which surra has been found in the wild deer in this island, in spite of their susceptibility and the constant presence of the virus in domestic stock. That it does affect the deer and rapidly kills them was shown by an outbreak in a tame park herd in 1910. In this outbreak, out of 19 head, 16 infected animals died within a period of two months from the initial infection. Owing to the localised habit of the deer, it being rare for a beast to wander more than a mile or so from its natal area, it would appear that the condition is unlikely to become widely disseminated among them by their travelling about.

The possible mode of infection of the herd under consideration is of interest. *Stomoxys nigra* occur in enormous numbers in the forest, particularly during the hotter months, October to March, and are a veritable plague to all the larger mammals. A cart track runs through the infected estate and wanders across the clearing in which the infected herd usually feeds and where the sick animals were killed. It is probable that an infected ox travelling along this road supplied the virus, which could be readily transferred by the local *Stomoxys* to a neighbouring stag, as the latter would probably pay but little attention to a passing ox-cart in the close season. Once introduced into the herd it is easy to visualise, in view of the limited area in which the deer are concentrated to feed during a period of the day-time, how the numerous flies would have an opportunity of carrying the virus directly from a sick beast to any number of healthy ones in a very short space of time. Careful enquiry has failed to reveal the presence of any known sick oxen travelling along this road ; but, nevertheless, in view of the mild nature which the disease may assume in otherwise healthy cattle, it is not improbable that an infected animal may have used it, and we know that cases of the disease have been reported from cattle in that particular district during the present year. In addition, it is worthy of note that, in view of the measures in force for the segregation and treatment of infected stock, it is by no means unusual for native owners to conceal the condition and, where possible, eventually to have infected animals slaughtered and sold for food. Indeed, in view of the susceptibility, the frequently semi-tame state, and the large numbers of deer concentrated in relatively small areas in certain parts of the island through which infected oxen must habitually travel, it is remarkable that surra has not before been introduced in epidemic form among these game animals.

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AN EXPERIMENTAL STUDY OF THE "BENNETT AND KENNY" REACTION IN GUINEA-PIGS INFECTED WITH *TRYPANOSOMA EQUIPERDUM.*

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(*Received for publication* 10/6/33.)

It is known that certain anæmic or chronic diseases are marked by a tendency on the part of the globulins of the blood-serum to become unstable. Many non-specific reactions in serology are based on this fact. The most recent example of a reaction of this kind is that of Bennett and Kenny (1928), used in the diagnosis of the trypanosomiasis of the camel and later taken up by Fulton (1930) and adopted to the diagnosis of infective anæmia of the horse.

The reaction consists in obtaining flocculation and precipitation by allowing the serum to be tested to react with a very dilute solution of bichloride of mercury.

In order to determine the real sensitiveness of this test and to give it a strict experimental basis, we have carried out a special study upon guinea-pigs artificially infected with *Trypanosoma equiperdum.*

Since the guinea-pig does not naturally contract Dourine, we were in a condition to use for our experimental work as many certainly healthy animals as we needed, and to follow up step by step, from the day of the artificial infection to the very death of the animal, the gradual development of the reaction. Moreover, the subacute nature of the experimental infection of the guinea-pig by *Trypanosoma equiperdum*, which lasts for two or three months, has lent itself particularly well to our observations.

Technique Followed.

Collecting the Serum.—About 2 c.c. of blood are obtained from the ear of the animal by the zahn-aspiration apparatus, a method which is more practical than puncturing the heart ; the blood is left to coagulate at ordinary room temperature, and is decanted on the following day. In case of insufficient retraction of the clot it is carefully freed from the wall of the tube, centrifuged, and the serum decanted. Often there results a slight hæmolysis, tinging the serum rose, but this has no influence on the reaction.

These details may appear superfluous but are actually very important, as will be seen later.

Bichloride of Mercury Solutions and their Dilutions.—The solutions are prepared from chemically pure $HgCl_2$ and freshly distilled water. We have used the following dilutions : 1 : 1,000, 1 : 2,000, 1 : 5,000, 1 : 10,000, 1 : 20,000, 1 : 40,000, 1 : 60,000, 1 : 80,000.

Animals and Strains of Trypanosomes.—The guinea-pigs used were of both sexes and of weights varying from 300 to 700 gm. The strain of the

Trypanosoma equiperdum has been maintained in our laboratory for more than ten years by continuous passage in healthy animals, so that it became well adapted to its host species.

The infection of the healthy guinea-pigs was carried out by intraperitoneal inoculation of 3 c.c. of blood obtained from a strongly infected animal.

Reactions and Reading of Results.—To a series of test tubes each containing 1 c.c. of the aforesaid dilutions one drop of the serum to be tested was added, and the tubes were well shaken.

The reaction, which took place at laboratory temperature, was observed by light transmitted from a " Danox " lamp, and the results were noted at the second and twenty-fourth hours. When the reaction was positive, according to its intensity, a flocculation occurred, or an opalescence, more or less marked, followed by precipitation.

Sometimes the supernatant liquid did not clear up and very small particles remained in suspension.

Fig. I shows a positive reaction up to the 1 : 60,000 dilution of the $HgCl_2$ obtained on the second hour. It also gives an idea of the different degrees of flocculation and its subsequent precipitation, the intensity of which continues to diminish with the increasing dilution of the $HgCl_2$.

A control tube containing 1 c.c. of distilled water and one drop of the serum to be tested always accompanied the series of the other tubes.

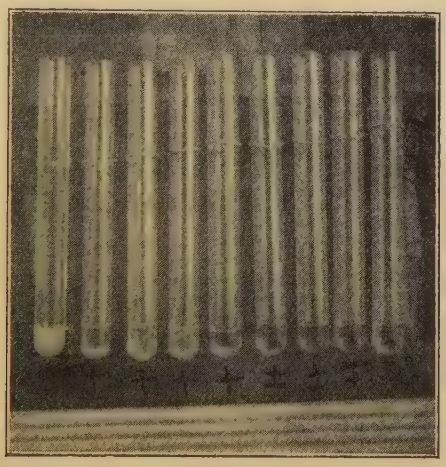

Fig. I.

The first tube indicates the positive reaction after shaking. The other tubes indicate the reaction after two hours. The dilutions are 1/1,000 for the first two tubes and for the other successively 1/2,000, 1/5,000, 1/10,000, 1/20,000, 1/40,000, 1/60,000, 1/80,000.

Flocculation Tests upon Healthy Animals.

In order to know the behaviour of normal serums, 60 healthy animals were tested. None of these showed, either at the first or at the second reading, any flocculation with solutions of $HgCl_2$ surpassing the dilution of 1 : 5,000. Fig. II shows a few of these reactions noticed among the healthy animals, and chosen because they represented the different types observed.

Flocculation Tests upon Infected Animals.

Twenty animals whose serum had previously been tested were inoculated according to the technique previously indicated.

About every tenth day the animals were weighed, their blood examined in search for trypanosomes, and the blood-serums subjected to the "Bennett

Fig. II.

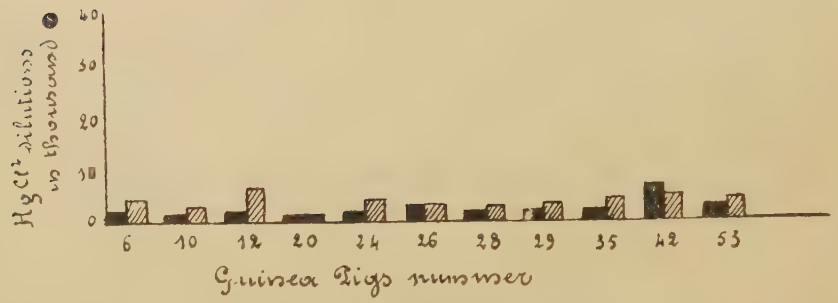

Fig. II.

Flocculence obtained with serums from healthy animals. The full line indicates reading on the second hour. The striated line indicates reading on the twenty-fourth hour.

and Kenny" test. The few graphs taken at random and represented by Figs. III and IV, demonstrate in a sufficient manner that very soon after the infection took place—usually in about 10 or 12 days, and also coinciding

Fig. III

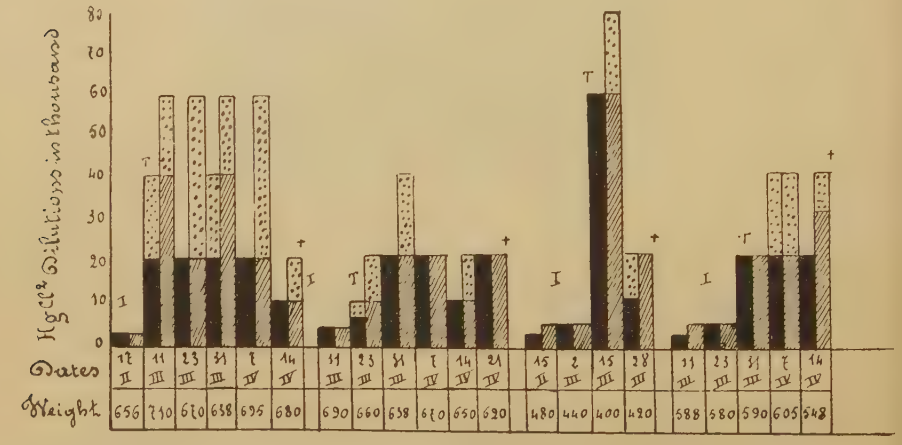

Fig. III.

Flocculence obtained with serums from four infected animals. Both lines signify the same as in Fig. 2. The punctiform areas represent a trace of flocculence. I —— Moment (time) of infection. T — First appearance of trypanosoma in the blood of the animal, + Death of animal.

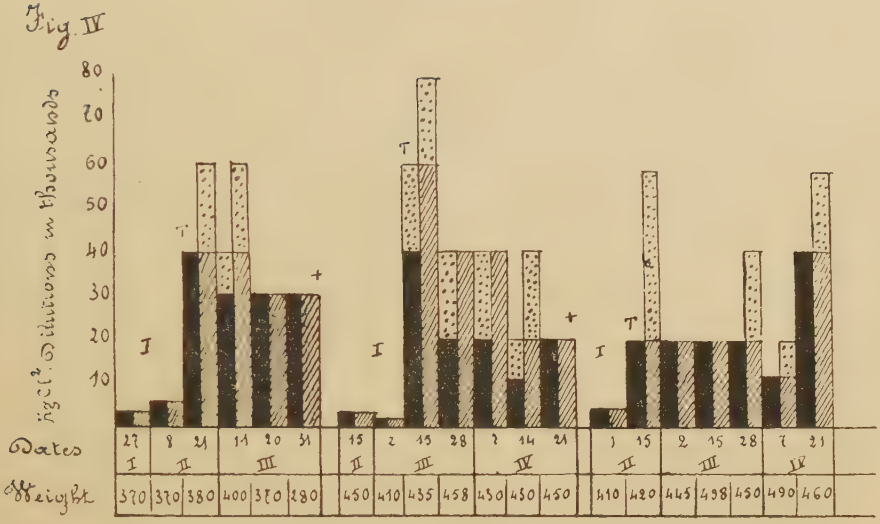

FIG. IV.

Flocculence in case of three infected animals.

with the first appearance of the trypanosomes in the blood—there is a strong elevation in the dilution rate of the $HgCl_2$ solutions that entail positive flocculation reactions.

Though very distinct at the first reading, when the reaction is already positive, even with dilutions of 1 : 20,000, the flocculence becomes still more evident and demonstrative on the second reading, which very often surpasses by 25 to 50 per cent. the results obtained at the second hour.

During the progress of the disease the reaction presents slight fluctuations but, whereas at the beginning of the infection the reaction tends to be positive with solutions more and more dilute, towards the end the reaction often becomes less characteristic (see Graph II, Fig. III), though never comparable with the normal. Finally, it will be noted that the difference between the healthy and infected animals is clear-cut. While normal serum does not flocculate with $HgCl_2$ solutions surpassing a 1 : 5,000 dilution, the serums of infected subjects always produce flocculation with solutions of 1 : 20,000 and in some cases even of 1 : 80,000 dilutions.

FACTORS THAT MAY INFLUENCE THE REACTION.

The " Bennett and Kenny " test, simple as it may appear to be, demands a certain degree of exactness in carrying it out, without which the clearness of the reaction may be lost. We have studied a few factors which tend to pervert the reaction, and which other authors have already partly indicated.

Collecting the Serum.—The serum removed too soon from the coagulum—after four to six hours, for example—with or without the help of centrifuging, produces reactions which are weaker than those given by serum collected 16 to 24 hours after the coagulation occurred ; furthermore, serum collected very early rapidly loses its power to flocculate (Graph I, Fig. V).

Hæmolysed Serum.—In spite of all precautions in collecting the serum, it often happens that certain specimens show hæmolysis. This fact, however,

appears to influence the reaction very little, as is proved by Graph II of Fig. V. Some of the serum-samples, perfectly clear, are divided into two lots, one of which is being utilised as it is, while the other is being subjected before use to an artificial hæmolysis produced by addition of fragments of clot previously ground (Graph II, Fig. V).

Preserving the Serum.—The serum gives its maximum reaction when collected on the eighteenth hour after bleeding the animal but afterwards it loses progressively its power to react if it is preserved at laboratory temperature. Similarly, if the serum is kept in the ice box (about 0° C.), its power to flocculate generally diminishes rapidly. However, some specimens show no apparent deterioration for four or five days and even more (Graph III, Fig. V).

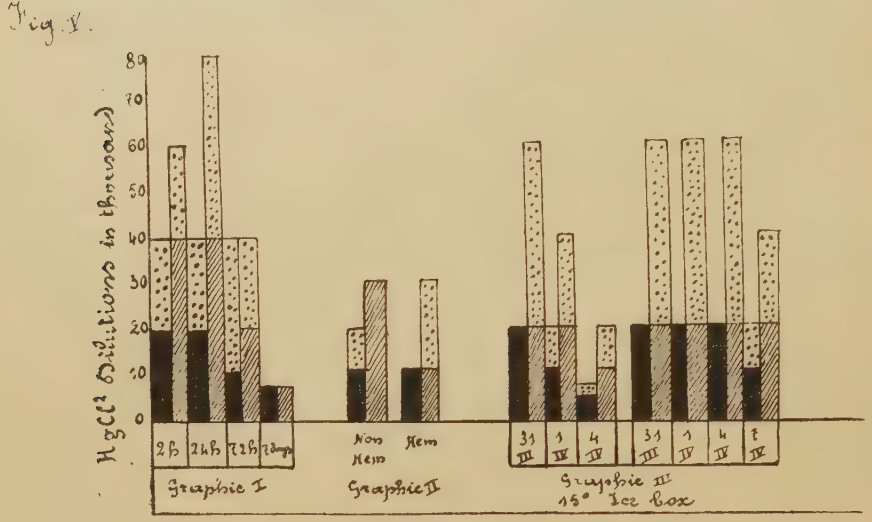

FIG. V.

Graph I represents the intensity of flocculence of the same serum, but removed from the clot and tested at different hours, namely, on the second, twenty-fourth and seventy-second hours and after seven days. Graph II. Influence of hæmolysis on the reaction. Graph III represents flocculence reactions of a serum, one part of which has been kept at room temperature (15°) and the other in the ice box.

Reading the Results.—During the reading one does not take any account of the very fine granules which settle on the walls or bottom of the test-tubes. The same kind of sediment is noticed, especially in the case of serums having a high power of flocculation, in the presence of distilled water, especially when the latter is fresh and consequently free from $HgCl_2$.

CONCLUSIONS.

The experimental study of the " Bennett and Kenny " reaction upon guinea-pigs artificially infected with *Trypanosoma equiperdum*, has allowed us to appreciate in a strictly accurate way the diagnostic

value of this method, which is at the same time clear and reliable, and gives early results. With the first appearance of the *Trypanosoma equiperdum* in the blood of the host, *i.e.* 10 to 15 days after the infection, the reaction tends to rise a little and then stabilises itself, without ever failing, even during the stages of crisis, at which time no trypanosomes are to be found in the blood of the subjects.

Although it does not possess any specificity, we think, however, that this method of serological diagnosis will be able to render great services, not only in the matter of discovering camel trypanosomiasis, but in all cases of animal trypanosomiases, with one condition only, namely, the previous study of the modifications that may be necessary for each different species.

It is possible that Manley's conclusion that the "Bennet and Kenny" test was not serviceable for the diagnosis of trypanosomiasis in the ox and goat was the result of a too rigid application of the method to these animals.

It affords me pleasure to express here my sincere thankfulness and high appreciation to Prof. van Goidsenhoven, chief of our laboratory, for his guiding criticism in this work, and to Dr. Bertrand, fellow assistant, for his friendly guidance and help.

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THE CONTROL OF CAMEL TRYPANOSOMIASIS.

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(Continued from page 77.)

Immunity following Recovery.

It was concluded by Knowles (l.c. 1927) that " Camels cured of trypanosomiasis by Naganol have a definite immunity against the disease, which immunity is of long duration." The protocols do not support this conclusion, which was drawn from the observation of three camels only. Of those, the first (No. 1,052) had been treated with three doses of Naganol, each of 6 grammes, at intervals of two days, and had been completely cured. Between nine and ten months later a test dose of infected blood was injected ; trypanosomes appeared in the blood and remained there until the camel was destroyed, and a positive formol-gel reaction developed. Two control camels developed a more severe infection. The tested camel is recorded by Knowles as having had a " relative immunity " of nine and a half months' duration. The second camel (No. 1) had been given 6 grammes but had relapsed 11 weeks later and had then been given an additional 10 grammes ; a test injection of trypanosomes nine and a half months after the last dose was withstood. The third camel (No. 1,630) was cured with a single dose of 10 grammes, and when tested six weeks later trypanosomes appeared in the blood for a few days, but subsequently disappeared, and the camel thereafter remained apparently healthy.

The first case, in the writer's own estimation, should have been regarded as having no immunity whatsoever ; that two control camels developed a more acute infection is of little interest, since one has observed that on infecting groups of clean camels with a single sample of blood hardly any two develop symptoms of equal intensity. The second camel was " immune," but was not a fair case to quote, since cure of a primary infection was not at issue. The third camel had an immunity, but this was not of very convincing solidity even at so short an interval as six weeks after cure. It also has to be pointed out that the purely prophylactic qualities—if any—of the Naganol were not taken into consideration.

Further evidence was obviously required, and since it is neither easy for the laboratory to keep sight of many cured camels, nor for officers in the field to carry out immunity tests, complementary observations in the laboratory and field were undertaken.

In the laboratory two recently-cured camels were tested early in 1927, one of which had been cured with a single dose of 10 grammes of Naganol, and one with 3 grammes of Naganol and 2 grammes of sodium antimonyl tartrate. Tested at seven and six months

respectively after cure, both proved to be immune. A more systematic test was then undertaken. Late in 1927 twelve working camels were infected and allowed to continue in work until they were losing in efficiency and had to be treated. Each one coming up for treatment received a single intravenous dose of 10 grammes of Naganol and all were cured. The further object was to test two or three as to immunity at various intervals. Actually two tests only were carried out, at four and at eight months after cure, and the results were as follows :—

(*a*) *Four Months after Cure.* Two camels tested with one control (April 10th, 1928). The control camel developed trypanosomiasis, the mercuric chloride reaction became positive, and the camel had to be treated. One test camel remained negative throughout, and two white rats injected with its blood two months later remained healthy ; the other test camel remained apparently healthy, but two months later one of two white rats injected with its blood developed trypanosomiasis ; at an interval of a further two months rat inoculations proved negative.

(*b*) *Eight Months after Cure.* Two camels tested with one control (August 6th, 1928). All three developed trypanosomiasis which persisted and had to be treated. Mercuric chloride reactions also became positive in all three cases. It would have been instructive, at this point, to have tested the immunity of the remaining cured camels of the original 12, but the official in charge of the unit, having seen the result of the test on two camels, would not agree.

The result of the foregoing test in spite of its being incomplete was sufficiently definite. Even four months after cure, one of two camels, although sufficiently resistant to throw off the reinfection, was not completely immune, while eight months after cure neither tested camel had any resistance whatsoever.

In regard to field observations, a single 10 gramme dose was still being retained as the routine treatment, and the retention of this large dose ensured that all subsequent infections in treated cases would be reinfections and not relapses. None but officially-owned camels were being treated and the later history of all of these, except such as died outside veterinary observations, was available for study. Actually between 500 and 600 records were studied, and from them it was ascertained that at any period longer than six months after cure reinfections were common, a few were recorded after four or five months, and only a single case after three months. In assessing this evidence, which has since been confirmed by rather less intensive observations on some thousands of camels over a period of four or five years, it has to be strongly emphasised that, in consideration of the seasonal incidence of trypanosomiasis, any period between about four and eight months after cure lies in a season during which the chances of reinfection are at their lowest, and the recording of even a small number of reinfections implies that the susceptibility rate is really high.

Examination of the foregoing evidence necessarily leads to the conclusion that the period of immunity following recovery after Naganol treatment is relatively short. The " bracket " of four/eight months established in the laboratory develops as the result of field observation into an average period of five or six months. It is particularly to be noted, however, that a five or six months' period of resistance is not universal ; reinfections may occur earlier.

The camels providing the foregoing data on immunity had all been treated in the relatively early stages of infection ; those under direct laboratory control were treated as soon as definite clinical symptoms developed, and the field cases, although inevitably in varying stages, were all treated immediately following the establishment of a positive diagnosis. Treatment in the earliest possible stage of infection is always the aim in general practice, and an immunity of short duration is what will normally be observed. It was, however, considered possible that a more lengthy immunity might remain in camels allowed to proceed to a more advanced stage of the disease before being treated. Two such cases were, therefore, studied in the laboratory, both of which were allowed to remain untreated until almost moribund. One of these when tested as to immunity ten months after cure proved resistant, while the other, tested 13 months after cure, proved susceptible. That one of these was immune 10 months after cure, whereas the average period of resistance is only five or six months, probably indicates that some degree of specific immunity may develop provided the patient lives long enough, but the susceptibility of the other camel 13 months after cure equally indicates that such immunity is not of a very high order. While of interest from the purely immunological standpoint this tentative conclusion is of no practical value, since it could never be suggested that for the sake of a more durable immunity all infected camels should be allowed to proceed to an advanced stage of the disease before being treated.

The development of a true immunity is thus not necessarily denied, but the following section dealing with the prophylactic properties of Naganol must be considered, in order to appreciate " immunity " in relation to all relevant factors.

Prophylactic Value of Naganol.

Mention has been made in the preceding section, of the early neglect to consider the possible protective period conferred by Naganol alone, when studying the duration of immunity in cured camels. The point called for investigation, although in consideration of the short period of resistance following cure it did not seem that any result worthy of applying to general field practice would be obtained. Having found by the study of two chronic cases that some kind of specific immunity might possibly develop, it seemed that such immunity would be at least as likely to account for the few months' insusceptibility to infection of average cured cases as

would residual traces of Naganol. It had, however, already been shown by Yakimov and his collaborators (1927), with a similar kind of camel trypanosomiasis in the Ural region, that in the face of natural infection a single dose of 5 or 10 grammes (details not very clear in the abstract consulted) would protect healthy camels for at least three months.

In designing a preliminary experiment in the laboratory the field for investigation was thus limited in extent. Existing evidence showed that, in point of dosage, 10 grammes (and possibly 5 grammes) of Naganol would protect healthy camels for three months, while the protective period conferred by the double influence of 10 grammes, plus whatever specific immunity might develop, did not in average cases extend to more than five or six months. It was considered advisable to test the prophylactic power of a " small " and a " large " dose of Naganol ; actually 4 grammes and 10 grammes respectively were used.

Outline of Experiment (November 1st, 1931).—Twelve camels giving negative reactions to the mercuric chloride test were purchased. Three weeks later the negative reaction was re-checked, blood being injected into gerbils (desert mice, highly susceptible to trypanosomes), which subsequently remained healthy. Six camels were given 4 grammes of Naganol and six were given 10 grammes. The further programme was to inject two of each group and a control with virulent blood at intervals of two, four and six months. The camels immediately before testing were to be checked as to freedom from infection by applications of the mercuric chloride test and by gerbil inoculation. After the injections of virulent blood they were to have their blood examined daily, the mercuric chloride test being applied weekly, and any camel remaining negative in both cases for a period of two months was to have samples of its blood injected into gerbils. (One of the 4-gramme camels died of intercurrent disease, so that at the sixth month one only of this dosage came up for test. As will be seen, this did not make any difference, since the protective value of 4 grammes had been determined much earlier). A summary of results is as follows :—

(*a*) *Test two months after giving Naganol* (January 22nd, 1932).—Camels Nos. 24 and 27 (10 gm. dose), 29 and 37 (4 gm. dose), and L. 25 (control), were all confirmed negative to the mercuric chloride test, and blood from them injected into gerbils, which subsequently remained healthy. All were given infected blood. Both 10-gramme camels resisted infection ; their blood remained free from trypanosomes, their mercuric chloride reactions remained negative, and gerbils injected with their blood two months later remained healthy. Of the 4-gramme camels, No. 29 remained free from infection according to the standards just described ; No. 37, however, developed at the fourth week a positive mercuric chloride reaction which continued to intensify, and on the thirty-fifth day trypanosomes appeared in the blood. The camel continued to lose condition,

with trypanosomes frequently visible in the blood, and had to be treated. The control developed trypanosomiasis in the ordinary way.

(*b*) *Test four months after giving Naganol* (March 22nd, 1932).—Camels Nos. 30 and 32 (10 gm. dose), 31 and 35 (4 gm. dose), and L. 26 (control) checked for freedom from infection as above and all given infected blood. Both 10-gramme camels completely withstood infection according to the standards already described. Of the 4-gramme camels, No. 35 completely withstood infection, but No. 31 developed a positive mercuric chloride reaction at the fourth week, and trypanosomes appeared in the blood on the thirtieth day. The mercuric chloride reaction continued to intensify, trypanosomes remained almost constantly in the blood, and the camel had to be treated. The control behaved almost identically.

(*c*) *Test six months after giving Naganol* (May 28th, 1932). Camels Nos. 34 and 38 (10gm. dose), 36 (4 gm. dose), and 39 (control) checked for freedom from infection as above and given infected blood. To avoid unnecessary detail it may be briefly stated that all five camels developed trypanosomiasis which progressed to a stage at which treatment became necessary.

The results of this experiment call for little discussion. Four grammes of Naganol can obviously not be relied upon to protect for even two months, and while 10 grammes may protect for four months it is no longer protective after six months. The experiment definitely indicates that the period over which a healthy camel can be protected is proportional to the amount of Naganol given, although it would be rash to suggest that the proportion is arithmetical. Further, as shown by the variable behaviour of individual camels, it will be impossible to estimate with any precision the average or even the minimum period over which a given dose will protect without statistical treatment of large scale observations, and for immediate practical purposes this is not indicated.

A general consideration of camel trypanosomiasis contra-indicates the routine prophylactic administration of Naganol. In average seasons the incidence is from 15 to 20 per cent., although in bad seasons in bad localities it may exceed 50 per cent. With the present system of control by diagnosis and treatment very few camels die, and the expense of giving a sufficiently large dose of Naganol to ensure resistance over the normal annual period of exposure—if indeed resistance could always be ensured, which is not certain—would not be economically justified. There are, however, undoubtedly circumstances in which the procedure might be profitably adopted; for example, for the protection of police patrols or in military operations over a short period entailing the use of small units or the splitting up of units into bodies so scattered that veterinary supervision is temporarily impracticable. If such a practice were contemplated it would be advisable to determine the probable period of protection afforded by the maximum tolerated dose. This dose has not been definitely determined, but a few observations have shown that it will

probably lie between 20 and 25 grammes for an adult camel (ca. 7·0 gm./100 kg.)

Finally, it is necessary to compare the observations on prophylaxis with those on immunity. It then becomes evident that whether or not some specific immunity may develop -and the study of a few chronic cases indicates that the possibility may exist—in actual practice, in which camels are treated as early as possible in the course of the disease, immunity must be considered as non-existent. A short period of resistance to reinfection will follow cure, but this resistant period will be attributable to residual traces of Naganol, and its duration, while varying somewhat in individual camels, will in general be proportional to the dose of the drug originally given.

Trypanosome " Carriers " of Other Species.

The camel largely performs its duties without particularly close association with other animals, but it necessarily comes into some contact with other domesticated species, and on this account it is relevant to consider whether some of these may not act as " carriers " or " reservoirs " of *Tryp. soudanense*. It is worthy of note that up to the time at which it was decided to investigate this question no other animal species in the Sudan had ever been found suffering from natural infection with this trypanosome, although, of the common domestic animals, cattle and dogs had been experimentally infected and cattle had been found to recover spontaneously. It appears that the local strain differs in its natural pathogenic range from trypanosomes of the same type in other countries.

Limiting one's considerations to horses and donkeys, which in the Sudan are more closely associated with camels than are other domestic species, all that has been done in the case of the former has been to attempt artificial infection, partly with the view of ascertaining whether such infection was possible but mainly with the object of studying any symptoms that might develop, in order to judge therefrom whether natural infection, if it occurred, would be likely to attract clinical notice. In the case of donkeys, owing to their low cost, more extensive observations were designed, the series of investigations being :—

(*a*) Is it possible to infect donkeys with the local strain of *Tryp. soudanense* ?

(*b*) Is the course of the disease of such a nature that it might escape clinical notice ?

(*c*) Do infected donkeys, if susceptible, recover spontaneously ?

(*d*) Is it possible to cure them easily—the therapeutic agent in mind being Naganol ?

(*e*) Is it not possible that a more careful search will reveal naturally ocurring cases ?

In regard to horses, one horse only was injected with infected blood from a camel. Trypanosomes appeared in the blood on the fourteenth day and remained more or less continuously, the horse

dying after six months. It was thus provisionally concluded that if horses did become infected in nature the infection would be noticed, and that as no infected horse has yet been seen in the Sudan natural infection does not occur. The horse in fact does not constitute a danger to camels.

In regard to donkeys the case was otherwise, as will be shown by the following analysis of observations :—

(*a*) *Possibility of infection* (November 12th, 1927).—Three donkeys were proved free from infection by white rat inoculations, and were then given blood from an infected camel. Trypanosomes appeared in the blood in each case on the seventh day.

(*b*) Course of infection.—Temperatures were taken twice daily and blood was examined daily. In all cases there were intermittent attacks of mild fever lasting three or four days, but at very irregular intervals. When there was fever trypanosomes were always visible in the blood, but they were also frequently visible when there was no fever. The donkeys fed well throughout and did not lose condition ; one of them was given regular light work and maintained condition quite as well as the other two. One of the donkeys was treated after five weeks, but the other two were kept under observation for seven months ; for ten weeks the symptoms continued as above, but after this point in both instances no trypanosomes were ever seen in the blood and there was no further fever or other symptoms of any kind.

(*c*) *Possibility of spontaneous recovery*.—When treating one donkey the two others were left as controls. As has been recorded in the preceding paragraph these, after the sixty-eighth and seventieth day respectively, showed no more symptoms. In order to ascertain whether they had recovered spontaneously samples of their blood were injected, 211 days after infection and 143 and 145 days respectively after trypanosomes had last been seen in the blood, into white rats. All of these developed trypanosomiasis.

(*d*) *Possibility of cure*.—One donkey, 39 days after original infection, and while in the active stages of the disease, was given 1 gramme of Naganol intravenously. Fever ceased at once and trypanosomes were never again seen in the blood. Five and a half months after treatment, blood was injected into white rats, which remained healthy. The other two donkeys, having served as controls to the first, were now treated with one gramme of Naganol, 161 and 163 days respectively after trypanosomes had last been seen in the blood (although white rat inoculation proved them to be still infected). No subsequent blood examinations were made, but two months later samples were injected into white rats ; all of these remained healthy.

(*e*) *Possible existence of natural cases*.—The general character of the artificial infection made it appear quite possible that natural cases might occur. That none had hitherto been brought to notice could be explained both from the general absence of symptoms and from the inability to detect trypanosomes in the blood in any but the early

stages. It was therefore arranged to examine a number of donkeys at Kosti (on the White Nile, about 200 miles South of Khartoum—a locality notorious for camel trypanosomiasis). Samples of blood from eighteen donkeys were examined microscopically (cover-slip preparations) and blood from fourteen was injected into white rats. No trypanosomes were seen and none of the rats became infected. Later in the same year (1929), at the end of the " fly " season, however, a natural infection in a donkey was detected (at Gedaref, Kassala Province—another notorious locality for camel trypanosomiasis). In this case trypanosomes were seen in the donkey's blood. The donkey was brought to the laboratory and kept under observation but trypanosomes were not subsequently seen, although white rats inoculated three and six weeks later all became infected. At the end of six weeks an intravenous injection of 1 gramme of Naganol was given ; white rats inoculated one and two months thereafter remained healthy.

The general conclusion regarding possible " carriers " amongst horses and donkeys may therefore be summarised as follows :—

In regard to horses, the artificial infection studied would indicate that if natural infections occurred they would come to notice. That none has so far come to notice shows that natural infection probably does not occur.

In regard to donkeys :—

(i) Donkeys are susceptible to *Tryp. soudanense*.

(ii) Natural infection occurs, although it may not be very common.

(iii) The course of natural, as of artificial, infection is such as would lead to the establishment of " carriers."

(iv) The infection, artificial or natural, appears to be easily cured, a single dose of 1 gramme of Naganol being sufficient.

(v) Arising out of the above, it will be a useful measure—in dealing with large camel units at any rate—to treat closely associated donkeys at the end of the " fly " season. The ease, cheapness and efficacy of this procedure will counterbalance the fact that it may in most individuals be superfluous.

The Tsetse-Borne Trypanosomiases in Camels.

It has already been mentioned that camels are not as a rule exposed to infection by the tsetse-borne trypanosomes, and on this account the latter have received little attention. The following few observations are nevertheless of interest, since the question of working camels in tsetse areas may yet arise.

Tryp. brucei.—The only record that can be found of natural infection of camels with this trypanosome in the Sudan is by Wenyon (1908), and on that occasion a high rate of mortality was observed. In the laboratory only one case has been studied. It was artificially infected with blood from a mule. Trypanosomes appeared in the blood within a week and were almost continuously present for a month ; a positive formol-gel reaction developed in seven weeks.

A single intravenous dose of 10 grammes of Naganol effected a complete cure.

Tryp. vivax.—The only observation has been of one camel experimentally infected with a bovine strain. Trypanosomes appeared in the blood on the seventeenth day and remained until the twenty-fifth day. From this point, although trypanosomes were not subsequently seen in the blood, the camel continued to lose condition. The mercuric chloride reaction became positive one week after infection and continued to intensify until the sixth week, at which point the camel was treated. A single intravenous dose of 10 grammes of Naganol effected a complete cure ; the camel recovered its normal condition and the mercuric chloride reaction became negative at the ninth week after treatment. Owing to the uncertain transmissibility of *Tryp. vivax* to experimental animals, the continued infection after the twenty-fifth day and the certainty of cure were not checked biologically ; the recovery of bodily condition and the disappearance of the mercuric chloride reaction after treatment may, however, be accepted as sufficient evidence of the latter.

Tryp. rhodesiense.—The human strain recorded by Archibald and Riding (1926) was kindly supplied by them. One camel was injected with infected rat blood. Trypanosomes appeared in the blood on the seventh day and continued present in large numbers, causing acute symptoms until the eighteenth day. Circumstances did not permit of more prolonged observation, and on the eighteenth day a single intravenous dose of 10 grammes of Naganol was given. A complete cure was effected, checked three months later by inoculating guinea-pigs ; these remained healthy.

Tryp. congolense.—Natural infection with this trypanosome was first seen in this country in 1927. It was found in the field to run an extremely acute fatal course, as judged by records of thirty odd camels. Two camels were inoculated in the laboratory, the intention being to treat one and retain one for further observation. The history of the untreated camel was :—

Zero day : Inoculated.

Nineteenth day : Trypanosomes in peripheral blood (and persisted in large numbers until death).

Twenty-seventh day : Camel off feed.

Twenty-ninth day : Swaying gait when made to walk.

Thirtieth day : Died.

There was continuous high fever from the first day on which trypanosomes appeared in the blood.

In the case of the treated camel the history was as follows :—

Zero day : Inoculated.

Thirteenth day : Trypanosomes in peripheral blood.

Fifteenth day : Off feed, trypanosomes swarming in blood.

Sixteenth day : Collapsed and became comatose, 10 gm. Naganol given.

Seventeenth day : Camel improved, trypanosomes few.

Eighteenth day : Camel apparently quite normal and trypanosomes absent.

Twenty-sixth day : Trypanosomes reappeared in blood.

Twenty-seventh day : Swaying gait, trypanosomes numerous, 20 gm. Naganol given.

Twenty-eighth day : Camel quite normal, trypanosomes absent.

Thirty-sixth day : Trypanosomes reappeared.

Thirty-seventh day : 10 gm. Naganol and 2 gm. Sodium Antimonyl Tartrate given.

Thirty-eighth day : Camel quite normal, no trypanosomes.

The camel continued apparently normal for 24 days, and then died from some undiagnosed cause.

Two conclusions can easily be deduced from the foregoing evidence, first that *Tryp. congolense* causes a much more acute disease in camels than the other trypanosomes, and second that it is more difficult to cure ; it is not even certain that the treated camel was cured.

In regard to the tsetse-borne trypanosomes it is thus evident that the camel is universally susceptible, but that with the exception of the *Tryp. congolense* type the infections are easily cured with Naganol. If it ever becomes necessary to work camels in tsetse areas it seems quite probable that the trypanosomiasis resulting from such a procedure could be controlled. Further study of *Tryp. congolense* infections would, however, first be strongly indicated.

Summary.

The control of camel trypanosomiasis (*T. evansi* type) is most easily effected by diagnosis and cure. In regard to diagnosis, the mercuric chloride test has shown itself to be of high accuracy under laboratory conditions, and although it may lose somewhat in precision under field conditions it is by far the best available method. It has the advantages that it develops in all infected camels, that it develops early in the disease, that it never disappears spontaneously, and that it always disappears following cure. The only faulty diagnoses appear to be due to the existence of a few positively reacting non-infected camels, and to the fact that under field conditions the technique may so suffer from lack of facilities as to permit of apparently positive reactions in healthy camels. Both of these factors have been found in practice to be of little account ; healthy camels giving a true positive reaction are extremely rare, and whenever their number is apparently slightly increased owing to the necessity for carrying out tests under bad conditions the only loss is a dose of Naganol.

Treatment is universally successful with a single intravenous dose of Naganol. The smallest dose that has been found successful over a long period in the field is 4 grammes, but it is not yet certain that a smaller dose may not suffice.

Immunity following cure is for practical purposes non-existent. Camels are certainly resistant to re-infection for a short time after

treatment, but the resistance in general practice may be considered as entirely due to residual traces of Naganol, since the resistant period, except possibly in advanced chronic cases, is no greater than that conferred by Naganol alone on healthy camels.

For prophylactic treatment Naganol is of some value, but in routine field practice its use in such a manner is not to be recommended, since diagnosis and cure constitute the best control procedure. The period of protection is proportional to the dose administered ; with the present routine dose of 4 grammes protection for so short a time as two months is not certain. Circumstances, however, are indicated in which Naganol might profitably be used as a prophylactic.

Trypanosome " carriers " among other domestic animals play little part in spreading camel trypanosomiasis. Of the two most closely associated species, the horse in the Sudan is probably never infected, but donkeys may rarely be so, and the infection is of a type that would lead to the establishment of carriers. Suggestions are made for dealing with this situation.

In consideration of the foregoing data, it may be simply said that for routine purposes the mercuric chloride test should be freely used, in particular during and at the end of the " fly " season. Any camel showing a positive reaction, either for the first time or at an interval of three months or more after earlier treatment, should be treated.

Fortunately for field officers, the seasonal occurrence of camel trypanosomiasis, and the fact that most routine treatments are undertaken towards the end of the " fly " season, ensures that few camels require a second treatment in one year.

Regarding the tsetse-borne trypanosomes, camels appear to be susceptible to all species. Infections rarely occur in nature because camels are rarely introduced into tsetse areas. With the exception of *Tryp. congolense* the infections appear to be comparable with the common one due to trypanosomes of the *Tryp. evansi* type, and the routine system of control would be applicable. If deliberate exposure of camels to tsetse is ever contemplated an antecedent study of *Tryp. congolense* infection will be necessary.

Acknowledgments.

Before the official adoption of any control measure it has always been submitted to large scale trial in the field, and in the carrying out of such trial observations almost every field veterinary officer of the Sudan Veterinary Service has at some time or other been unofficially asked to collaborate with the laboratory. This collaboration is hereby acknowledged with gratitude. For prolonged observations, usually carried out with considerable difficulty on experimentally treated camels, and for the carrying out of many hundreds of mercuric chloride re-tests thereon, I am particularly indebted to Capt. J. Going and Capt. H. B. Williams, O.B.E.

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EQUINE MELANOMATOSIS.

By Sir John McFadyean.

The subject will be dealt with under three heads :—

1. Clinical.
2. Morbid anatomy and histology.
3. Source of melanin in the normal body.
4. Nature of the pigment-producing cells in melanotic neoplasms.
5. Ætiology.

Clinical.

Under this head, there may first be mentioned the great frequency of the disease in grey horses—a feature of the disease which is the more remarkable because melanotic tumours are comparatively rare in horses of any other colour.

It has been calculated that about 80 per cent. of grey horses of either sex develop one or more melanomas if they reach old age.

Another remarkable fact in connection with the disease is that it is very rare in animals under adult age. I do not know of any recorded case of the kind in an animal under adult age, but one very malignant case in this country in a five-year-old mare was brought to my notice 45 years ago.

The following table, copied from van Dorssen's work, shows in a striking way the steadily increasing tendency to the disease after middle life.

Age.	*Number.*	*Number with Melanomas.*	*Percentage with Melanomas.*
Under 6 years	17	—	—
6 to 8 years	45	5	11
8 to 10 years	52	19	36
10 to 12 years	60	37	61
12 to 14 years	38	27	71
14 to 15 years	7	5	71½
Over 15 years	15	12	80

The tumours may develop on the body, head, neck or limbs, but by far the most frequent site of the primary tumours is the tail, on the under side near the anus.

The primary tumours develop in connection with skin exclusively, and not in connection with any of the internal membranes or organs.

In only a small minority of cases does the disease cause death or reach a stage which necessitates slaughter, but highly malignant cases, fatal in consequence of rapidly growing internal tumours, are not rare.

Sometimes in the region of the tail, or on the body near the root of the tail, there may be several tumours, which in size and appearance suggest that they are all primary in the sense that they have each had an independent origin.

As a rule—though this is only known by post-mortem examination—the nearest lymphatic glands soon become involved through metastasis by way of the lymphatic vessels, and in the same way, at a later stage, the disease sometimes involves the peritoneum. A most extensive formation of tumours may be formed on that membrane, and similar growths may be present on the pleura. The spleen, liver, and lungs may all contain multiple tumours. The distribution of the tumours in these cases has a certain resemblance to what one sees in widespread tuberculosis in cattle. At first, the spread of the disease is mainly by way of the lymphatics, but the final involvement of the internal organs is probably mainly by way of the blood stream.

The growth of primary tumours is usually slow, but they may attain a large size, up to several pounds in weight. The skin over a tumour may ulcerate, but as a rule it remains intact. The consistence of the tumours is firm or almost fibroid, but the metastatic growths in the internal organs are much softer and sometimes almost diffluent. Section exposes a smooth, and in the denser tumours somewhat glistening, dark, almost black surface, with at most only a tinge of brown. As a rule, the whole substance of the tumour has this black appearance, but there are rare cases in which part of the tumour appears to be free from pigment or to contain very little of it.

The growth of the tumours appears to take place throughout their whole substance, and they tend to retain a rounded shape. There is little tendency to form a fibrous capsule, and the growth may merge into the adjacent normal tissue.

This tolerance of the tissues towards the melanin-forming cells is a striking feature of the tumours and may, perhaps, be considered a point in favour of the view that they are mesoblastic in origin.

Morbid Anatomy and Histology.

It has already been said that the substance of these tumours is generally black, and on microscopic examination of sections the histology of the growth is often obscured by the great abundance of melanin.

The accompanying micro-photographs, Figs. 1 to 4, show the varying amount of melanin in different tumours or in different sections from the same tumour. For the most part, it is contained in cells, some of which have giant dimensions. In addition to these melanophores, there is a varying amount of intercellular substance or stroma, which also contains scattered melanin.

A careful study of this stroma at different places in a section,

especially in one that includes the peripheral part of the tumour, shows that it is not a reaction product, but the remains of the tissue invaded by the tumour cells. It is itself more or less deeply

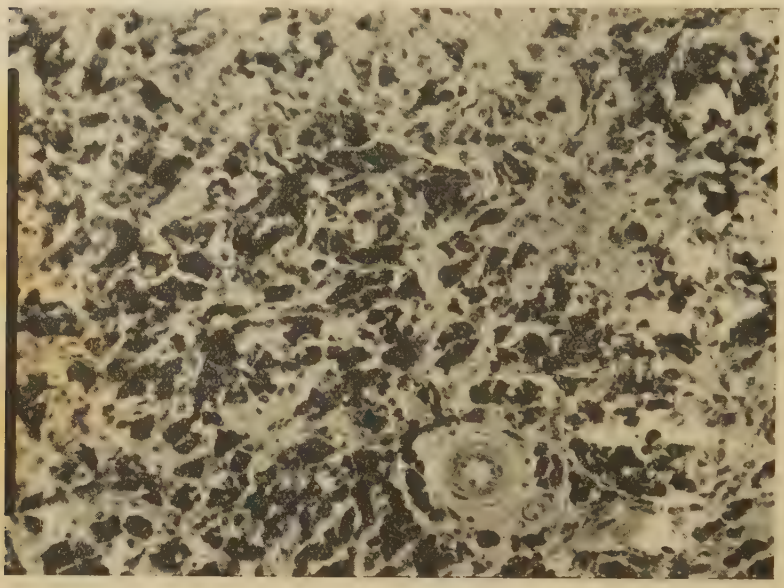

Fig. 1

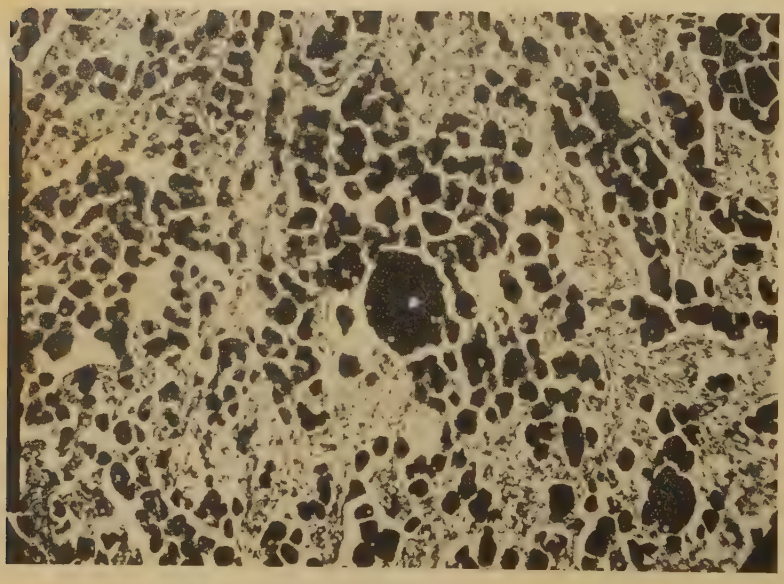

Fig. 2.

pigmented with scattered granules of melanin, apparently set free by dissolution of melanophores.

It is the large cells filled with melanin that naturally attract the

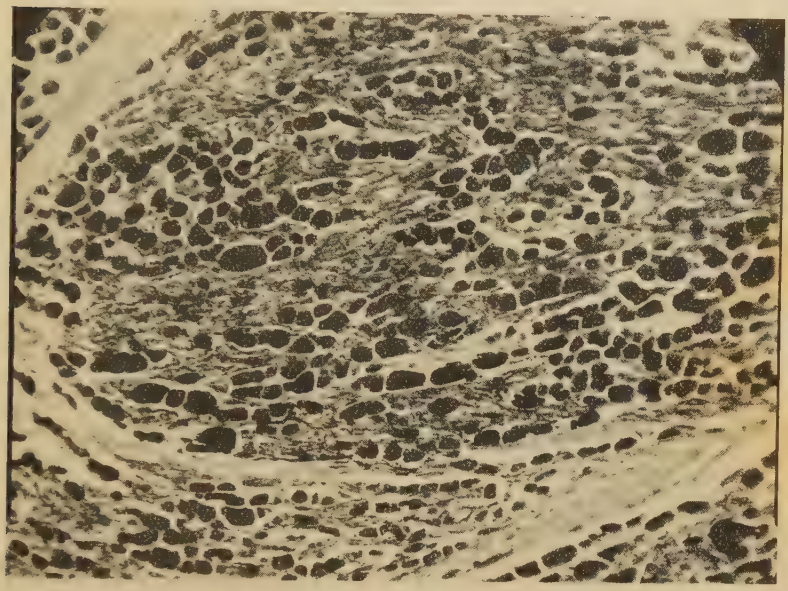

FIG. 3.

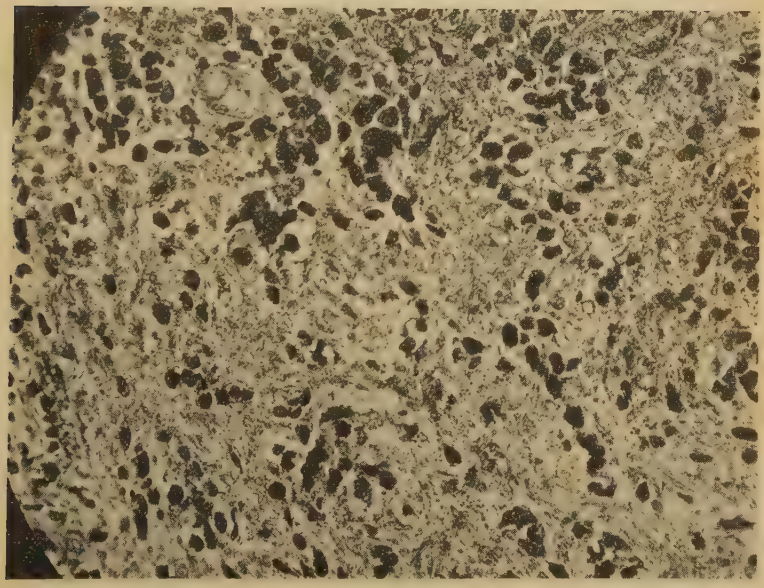

FIG. 4.

eye in a section, but it can hardly be believed that these are capable of either direct or indirect division in view of the disproportion between the cell contents and the remnant of cytoplasm. A melanoblast cannot be identified with certainty until it contains melanin, but there are found at some places groups of small cells without pigment which appear to be the young melanoblasts (see Fig. 6). The smallest cells containing melanin granules may be either round, oval, or spindle-shaped.

A better understanding of the histology of a melanoma is obtained from carefully bleached thin sections. The result is shown in Figs. 5, 6 and 7.

In the bleached and stained sections it can be seen that each cell has a nucleus, which may appear central but is in reality placed near the surface of the cell.

Some of the giant cells appear to have been formed by fusion, but others simply by independent growth. Ten or more nuclei may be present in one of these cells.

It will be noticed in Fig. 7 that the bleached cells have a resemblance to adipose cells, a fact that will be referred to later.

I am unable to agree with van Dorssen[1] that the equine melanoma should be classified as an alveolar sarcoma, and it is certainly not any kind of a carcinoma.

Source of Melanin in the Normal Body.

According to Robert Bonnet[2] the pigmentation of the skin and its epidermal appendages begins intra-uterine in the different animals at the time of the formation of the hair roots, through the wandering into the cutis of black pigmented cells or melanocytes, which, from there penetrate into the epidermis (prickle-cell layer of the hair bulb and same layer of the hoof horn), and here degenerating, give up their pigment to the epidermis cells, in which it is then stored up around the nucleus, mostly at its distal side.

Ellenberger and Gunther[3], in their "Comparative Histology of the Domesticated Animals," Third Edition, 1908, under the head of the fixed true connective tissue cells, say "The pigmented connective tissue cells, which occur only at a few places, are spindle-shaped, stellate, or irregular in form, are of considerable size and often provided with long processes which are frequently branched. The cell body contains in large amount pigment granules, so that it appears brown or black, whereas the nucleus as a rule appears as a clear speck."

Another paragraph headed, "Pigment of the Skin," is as follows:—"The epidermis, and frequently also the cutis, in our domesticated animals, with the exception of albinos, is pigmented diffusely or in spots (fleckig). The pigment granules lie partly in the pigmented cells of the cutis, and partly between the cells of the deep epidermis layers; the deeper they lie the more strongly are they pigmented. In the higher layers the number of pigmented granules is diminished; frequently one even finds a diffuse pigmentation of the cell body which can reach as far as the stratum corneum. In most cases, however, the latter is free from pigment."

Fig. 7

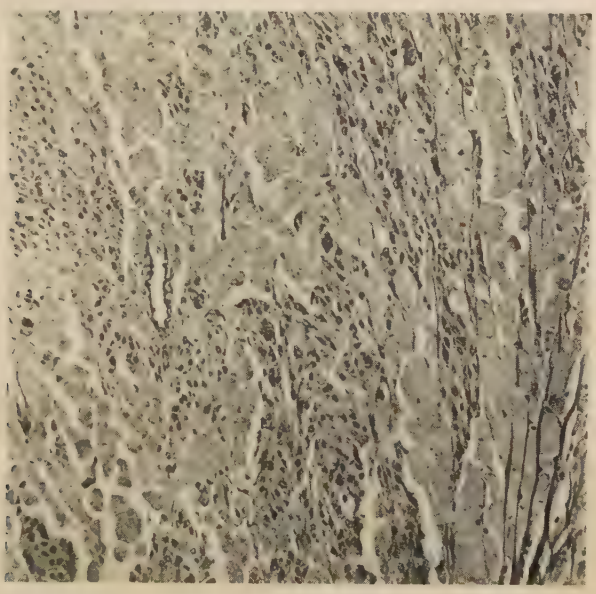

Fig. 6.

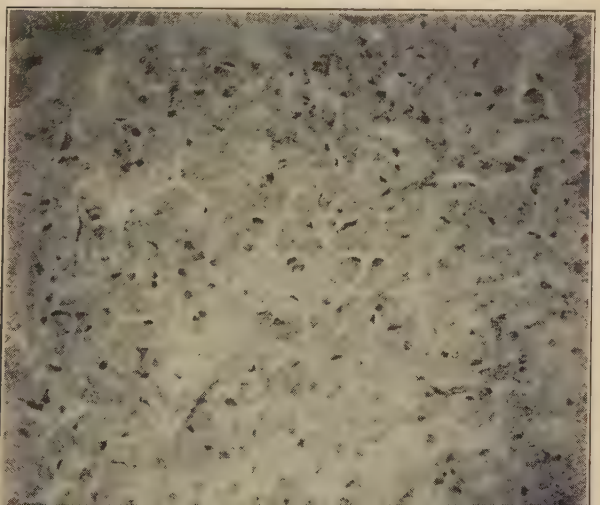

Fig. 7.

The following occurs as a footnote to the above paragraph :—" The skin is thus a pigment-destroying organ. Whether the pigment is derived from the epidermis or is carried into it by wandering cells from the corium is still uncertain. It is, however, certain that epithelial cells can furnish pigment, *e.g.* the epithelial pigment of the retina."

In Wells's " Chemical Pathology,"[4] the pathological formation of melanin is said to be mainly the result of an excessive production of the pigment by cells normally forming it, as in freckles, melanotic tumours, and perhaps in Addison's disease. Cells that do not normally form melanin probably do not acquire this power in pathological conditions. Pathological failure to form melanin is observed in albinism, in which the failure to form melanin may be attributed to hereditary influence. The skin formed in the healing of large wounds may also be devoid of pigment.

Tumour melanin does not differ from melanin produced by normal cells in any respect. Usually it contains much sulphur, even as much as 10 per cent., but the quantity of sulphur varies greatly in different tumours.

Although the amount of melanin in the entire skin of a negro may not exceed 1 gramme, Nencki and Berdez obtained 300 grains of melanin from one sarcomatous liver, and estimated that in that case the entire body contained 500 grains. According to Helman, melanin may constitute 7·3 per cent. by weight of the fresh substance of some melano-sarcomas.

Gustav Riehl[5], in his article published in 1884, is concerned principally with the pigmentation of the hairs. His own investigations had led him to certain conclusions for which he did not claim general acceptance, as hitherto he had only examined the hairs of the head, beard and eyelids of the human subject.

According to his experience the hair pigment was always intra-cellular in the papilla and matrix of the hairs, mainly in wandering cells, and in the horny part of the cortex always united with the protoplasm of the epithelial cells.

In transverse sections through the hair papilla at the place of its greatest circumference one finds in the papillary tissue of the growing hairs irregular-shaped cells with granular and strongly pigmented protoplasm, without distinct nuclei. The cells are partly round and partly spindle-shaped, or they have quite irregular outlines—pigmented wandering cells. The amount of pigment varies considerably in the different cells.

The way in which the pigment passes from the branched wandering cells and their outrunners into the epithelium could be followed in thin cross or longitudinal sections of hairs of the head or beard. When one follows the processes of the pigment cells one frequently finds some of which the part next to the cell body has a sharp contour, and is sharply defined towards the clear epithelium, whereas its end is indistinctly marked, either through a pale seam, or is quite unrecognisable. In the neighbourhood of such processes one always finds in the protoplasm of the cells of the hair cortex, diffuse yellowish-brown staining, and pigment granules in greater or less amount, and often heaped up, only at the surface turned towards the process, so that one gets the impression that the process became fluid and was taken up by the epithelial cells. This picture recalls sharply the way in which coloured particles are taken into their protoplasm by amœba from the surrounding water. However, the author left it undecided whether in these processes the cytoplasm was passed over along with the pigment. He

was not able to find with certainty free pigment at any part of a hair between the cells.

The article is accompanied by a lithographic plate containing two figures, one of the transverse section of a hair root, and the other of a longitudinal section. Both of these show pigmented, branched, so-called wandering cells, present both in the hair papilla and between the epithelial cells of the hair cortex.

Aeby's researches (1885)[6] were made on the skin of man and other mammals, and the fowl. He thought that all the results thus obtained might be summed up in the single sentence, " No pigment is formed in epithelium."

Wherever it appears in epithelium that is by way of wandering cells from the connective tissue. The wandering cells which serve as carriers may be present in great numbers. When insinuating themselves between the epithelial cells they change their shape according to the existing conditions, and in adaptation to the environment. They may remain rounded, or become elongated to form more or less thread-like structures. Their substance is always situated in the intercellular spaces of the epithelium, and very often it there forms an exceedingly delicate net of pigment. The farther outwards they penetrate in the epithelium the greater is the loss of colour of the pigment cells. Their substance becomes diffuse, the hitherto connected threads and nets are lost in a quantity of small particles which are absorbed by the epithelial cells. Preferably this pigment is deposited in the distal half of the cell, that is the end furthest from the connective tissue. The picture is often very characteristic owing to the fact that all or the great majority of the nuclei appear surrounded on one side by a dark half-moon of pigment. According to the extent of the progress of this process the intercellular substance gradually becomes free from pigment and clears up.

When the penetration of wandering cells is very extensive some of them may wander in more or less unchanged condition through the whole thickness of the epithelium. They escape destruction.

It is not to be assumed that the pigment-containing wandering cells differ in their nature from the pigment-free. The readiness with which one can recognise the ingestion of the former in the epithelial cells gives a finger sign. The pigment-carrying cells supply nutrient material to the epithelium that is becoming horny.

Ehrmann[7] (1885), who had studied the histology of the skin in frogs, reptiles, man and other mammals, rejected as absolutely without foundation the view that pigment is ever formed in the epidermis. The only question remaining to be examined was whether there are cases in which the pigment found in the epidermis cannot have moved upwards from the corium. In the amphibia all the stages appeared as so many phases of pigment ascent from the corium. There was no picture of pigment distribution which could be explained otherwise than on the assumption that the pigment was formed in the corium and moved upwards into the epidermis.

If one examines the skin of white people (at certain places), or of negroes, or pigmented animal skins, one always finds that pigmented connective-tissue cells are present in the corium. In the hedgehog and the bat Schoebl found branched pigment cells in the chorion united to form a rich network, just as in the amphibia. In men certainly the chief mass of the pigment is situated in the epidermis. This fact is to be explained by the fact that the provision of pigment by the corium to the epidermis is continuous, and

that nevertheless the pigment in the epidermis does not accumulate because it is not produced there and is cast off with the horny cells.

The epidermis cells are able to retain the pigment firmly and permanently, whereas the connective tissue cells do this only for a short time, and when the upward course of the pigment at any point in the corium is hindered, the pigment is taken up by the surrounding cells, and is subject to resorption.

The article is accompanied by excellent lithographic plates in colour. These include sections of the skin of the frog, salamander, conjunctiva of the ox, human skin, pigmented hairs and white hairs of Canities præmatura, the latter showing complete absence of pigment-carrying cells in the matrix of the hair.

Ten years after Ehrmann's article (1895) Hermann Post[8] published the result of his researches regarding the formation and distribution of melanin in the skin. His views were as follows :—

In the white skin of the human subject one finds here and there, singly or in small groups, pigment-containing cells in the epidermis, which mostly belong to the basal rete cells. Sometimes one sees in the neighbourhood of these single pigment-carrying cells in the connective tissue.

Brunette skin, and strongly pigmented parts of the skin, have an amount of pigment corresponding with the darkness of the colour of the skin ; the basal rete cells are all more or less pigmented, and the cells of the higher epidermis strata also contain pigment which quickly diminishes in quantity according to their distance from the rete cell layer.

The distribution of the pigment in the cells themselves is remarkable. The pigment is mainly deposited at the pole of the cell turned away from the connective tissue, there thus being formed the so-called distal cap. The amount of pigment in the connective tissue nearly corresponds with that in the epidermis. It lies in cells which, according to the place and the number, vary greatly, and are not essentially different from the unpigmented connective-tissue cells.

In the very dark negro skin the epidermoidal pigment is in much larger amount, but not the same everywhere. The author did not find branched pigment cells in the epidermis. The amount of pigment in the connective tissue was very nearly equal to that in the epidermis, but there were numerous connective-tissue pigment cells present which preferably followed the course of the blood vessels and were mostly found at some distance from the epidermis ; however, even in the immediate neighbourhood of the latter there were recognisable pigment cells. The connective-tissue pigment cells were irregular in respect of form and size, but they had not the shape of the branched pigment cells of the epithelium. Pigmented or unpigmented connective-tissue cells with a part of their body in the connective tissue and another part between the basal cells were never observed nor were they seen in the epithelium.

In the pigmented skin of guinea-pigs, cats, dogs, and cattle, Post always found branched pigment cells in the epidermis. These sometimes showed uncommonly long ramifications which could be followed for a length equal to the diameter of eight cells. These branches ran mostly parallel to the surface of the skin. The rete cells also contained more or less rich pigment. Of interest was the fact that, contrary to what is found in the pigmented human skin, pigment in the connective tissue was very sparingly present.

Since Post found the rete cells of the epidermis pigmented without the presence of branched pigment cells, and the rodlet form of the pigment made its source from the connective tissue improbable, he held that one

must recognise the power of the basal rete cells to form pigment. It was not necessary to assume that the cells of the higher layers of the epidermis, which always contain pigment in less amount than the basal cells, also form pigment, since they are the descendants of the basal cells and can derive their pigment from the latter.

Post thought that his researches appeared to justify the following conclusions :—

(1) The pigment of the epidermal structures arises in the protoplasm of the epidermal cells.

(2) Branched pigment cells develop in the epidermis from the ordinary epidermal cells.

(3) Where branched pigment cells appear in the epidermis pigmented connective-tissue cells are frequently absent.

(4) The basal rete cells possess the function of forming pigment.

(5) Pigment can pass over from the epithelium into the connective tissue.

(6) Pigment may be found in the connective tissue without the presence of pigment in the epithelium of the place.

Thus, pigment can be formed in the corium as well as in the epidermis, and in both positions, although the form is different, the substance may be the same and be derived from a common metabolic product of the skin. It follows that, if one presupposes a pigment-forming metabolic product, there is a sufficient explanation for the many manifold facts when one recognises that the formation of pigment varies according to individual local differences in the structure of the skin and the degree of thermal, chemical, or mechanical irritation.

At a place where the epidermis displays energetic formation of pigment, there will all pigment-forming substance be used, and the connective tissue will therefore remain free from pigment. This is the case in the development of the dark coloured feathers and hairs. In the pigmented skin the pigment formation in the epidermis cells is not so considerable. Here not all the pigment-forming substance will be used by the epithelium, and the pigment cells of the connective tissue will now act as useful regulators of the metabolism, since they can transform into pigment the substance to which the organism is certainly not indifferent, and convert it into an inoffensive material.

Under the head of the epidermis in the neighbourhood of melano-sarcomata, the author states that he examined two pieces of skin which had been excised as suspected metastases from a melano-sarcoma ; they were small pieces of blackish colour. About these pieces he says that, if it is permitted to draw any conclusion from the examination of such a small number of specimens, the first two sections appeared to show that a rich pigmentation of the connective tissue precedes the metastasis of melanotic tumours, and that there follows the formation of tumour cells, which on their part produce, by luxuriant proliferation, smaller and larger tumour nests. The pigment of the melanotic tumour therefore appears to contain a noxa which becomes diffused in the tissue and first causes a rich pigment formation, but later stimulates the cells to enlargement and proliferation.

The third preparation was interpreted to show that the epidermis cells are also influenced by the melanotic noxa, and can thus be induced to undergo extensive pigment formation and enlargement in size. What spoke in favour of this view was the discovery in some nævi of germ centres of melano-sarcoma, in the centre of which, at the boundary of the corium, there were found single or small groups of enlarged cells filled with reddish-yellow pigment.

Nature of the Pigment-Carrying Cells in Melanotic Neoplasms

Dr. E. H. Kettle[9], in " The Pathology of Tumours " (Second Edition, 1925), states that it is generally agreed that pigmented moles are derived from the chromatophores—the large pigment-bearing cells whose cytoplasm is prolonged into two or more processes—which are normally present in the cutis and the choroid of the eye. The author admits that the source of the melanin is still uncertain, and that we do not know whether it is derived from the hæmoglobin of the blood or is a degenerative product of the activity of the nucleus of the cell.

The author says, " The microscopic structure of many cutaneous melanomata suggests the correct origin of the chromatophores from the cells of the epidermis ; and the histological variations seen in some malignant melanomata are more compatible with an epithelial than a connective-tissue cell growth, but there is no general agreement in the matter, and the majority of authorities prefer to regard it as still *sub judice*."

Dr. G. W. Nicholson[10], after a careful study of the histology of the pigmented nævi or cutaneous moles of the human subject, is of the opinion that the nævus cells are of epithelial origin. They are, in fact, epidermal cells that have lost their fibrillation and prickles and are therefore no longer in organic contact with each other. In other words, they have lost an essential epithelial character, they are cast out from the epidermis and occupy the tissue spaces of the cutis.

The author refers to researches of Wieting and Hamdi regarding the source of the body pigment. These authors came to the conclusion that pigment is only formed by the epithelial cells. " All the cells of the rete malpighii can elaborate pigment, but certain cells, which are at first indistinguishable from the others of the basal layer among which they lie, become specialised in this direction. They are the melanoblasts (the chromatophores of writers), which are elongated, deeply pigmented cells with long branched pseudopodia. Pigment that is produced in excess is carried into the cutis by the lymph. It is here taken up by leucocytes and by connective tissue cells, many of which are branched. To the latter they limit the name of chromatophore. These cells store the pigment in the cutis or carry it away to the lymph glands, but never produce it. The epithelial origin of melanin is proved by its presence in the cells of the epidermis long before traces of it are found in the corium."

The author continues : " They [that is, Wieting and Hamdi] have definitely refuted Ribbert's view that the chromatophores of the corium are the primary producers of pigment, and that they enter the epidermis and transfer it to its cells, a view which, on purely *a priori* grounds, is a very unlikely one. Ribbert's argument that the nævus cells are mesenchyme cells thus falls to the ground. He believed them to be undifferentiated chromatophores."

Nicholson thinks that the nævus cells are degenerate. He quotes Dalla Favera to the effect that " they are degenerated cells produced by liquefaction of the intra-cellular fibrils and prickles of the rete malpighii, together with swelling and pyknotic nuclear changes, as a result of an excessive secretion of pigment. Before this pigmentary degeneration had set in these cells possessed the same morphological characters as their neighbours. They therefore show no signs of having remained in an undifferentiated condition."

" Pigmented moles are anomalies of position morphologically, since they are characterised by the dislocation of epidermal cells, the nævus cells, into the cutis."

Van Dorssen[1] (1903) came to the conclusion that in the normal skin of brown and black horses there are no pigment cells in the corium. In grey horses he found that the epidermis of the normal skin could be strongly pigmented, the melanin granules being present even in the stratum corneum. In grey horses, however, the pigment in the stratum granulosum was mostly found in the form of a cap on the distal side of the nucleus. In dark horses (black or brown) it lay more diffusely around the nucleus.

In sections of skin from the region of the tail and anus of twenty grey horses of medium or old age pigment specks were constantly found in the corium at the level of the sweat glands. When he had learned to recognise these spots under the microscope he could often detect them as punctiform black specks in the cutis with the naked eye.

In the skin of the lips and the ear such specks were sometimes seen, but in most of the pieces of skin examined from these and other places one could no longer speak of specks ; the pigment then showed itself here and there in the form of single scattered pigment cells.

The author had not had an opportunity to examine the skin of young grey horses ; the age of the animals whose skins he had examined was mostly over 12 years. However, he suggested that in young grey horses, just as in dark coloured horses, no pigment cells are to be found in the cutis, and that the pigment cells first began to appear in young grey horses when the foal coat begins to change into the proper grey colour.

Microscopic examination of the before-mentioned pigmented specks and streaks, with a higher magnification, showed pigment cells between the connective tissue fibres. The form of these cells varied to some extent—round, oval, three-sided, polygonal cells, branched cells with two or more shorter or longer outrunners, and all transitions between these cells ; some were decidedly stellate and then resembled the so-called chromatophores of the choroid.

Apparently free pigment was collected in the protoplasm of the outrunners. Here and there there was actual free, extra-cellular pigment between the fibres, probably originally intra-cellular pigment which had been made free by degeneration.

Further, there were long spindle-shaped pigment cells which lay close to the wavy connective tissue fibres. These fibres themselves contained more or less pigment, which was usually deposited in the long direction of the fibre. In consequence of this, and of the pigmented outrunners between the fibres, the specks often had a streaked appearance. A comparison of these pigment cells with the other cells in the connective tissue of the non-pigmented parts of the cutis showed that the pigment cells were different in form and size from the latter. The pigment cells are often larger and more variable in shape. The author regarded the above-named pigment cells as original, normally present, fixed connective tissue cells of the cutis, which under certain conditions, can take on a chromoblastic, in addition to their fibroblastic, function. In his opinion, every connective tissue cell in the cutis can become a chromatoblast or pigment cell.

The author affirms his belief that the cells in the pigment specks in the cutis of grey horses are normal fixed connective-tissue cells which have acquired the ability to form pigment. He says :—

" If we now summarise the results of my investigations regarding the skin of the horse—the unexceptional absence of pigment cells in the cutis of dark horses and the constant occurrence of these cells in the cutis of grey

horses of middle or old age—we see that these observations are completely in harmony with the opinion of Post on the occurrence or the absence of pigment cells in the cutis.

In grey horses, in which less pigment is formed in the epidermis, the connective tissue cells act vicariously as chromatoblasts."

To this pigmentation of the otherwise normal cutis the author applied the term " melanosis cutis," and he believed that in middle-aged and old grey horses it was the first step towards the formation of the primary melanoma of the skin.

The Primary Melanoma.—Van Dorssen was strengthened in his opinion that these pigmented specks marked the starting point of a melanoma when he often found distinct circumscribed submiliary and larger melanomas in the skin of grey horses.

Such intensively black specks differed in structure from the already described melanosis cutis.

In the latter the normal connective tissue was predominant, and the chromatoblasts were not so numerous between the fibres, while in the larger specks the pigmented elements, through marked increase, stood in the foreground, and the intervening connective tissue was diminished ; the normal structure of the cutis had there disappeared.

Under a higher power such a speck appears nearly like a pigment mass, but gives little explanation regarding the source of these proliferating pigment structures. In the many specimens examined various transitions from the microscopic small to the named larger specks were found. These transitions gave a distinct picture of the earliest stages of the proliferation. In the specks with chromatoblasts there were pigment cells which immediately attracted attention on account of their size and form and the large amount of pigment in them. After removal of the melanin these cells were round oval, often elongated oval, but always without outrunners or branching. In different preparations one could now find evidence of a multiplication of these intensely black cells, to form long rows or round irregular collections. The latter are surrounded by the connective tissue, which is bent outwards, compressed, and reduced to thin fibrils.

With a further multiplication of these cells the connective tissue diminishes and the pigment cells predominate in the microscopic picture. A striking fact is that in this stage of the growth the earlier chromatoblasts are no longer to be found ; they have gradually disappeared during the proliferation. Here arises the question, should these sporadically appearing large, round, and strongly pigmented cells in the specks of melanosis cutis be regarded as altered chromatoblasts, of which the already existing lively activity has been still more increased ?

The cause of this increased formative activity of the chromatoblast must be sought for in the special circumstances of the pigmentation of the hairs in grey horses as they become older. It is assumed that the first chromatoblasts in the cutis appear at the time at which the foals are exchanging their coat for the proper grey colour. After this exchange comes the mixed-colour coat. With increasing age the body hairs become increasingly lighter, and indeed frequently quite white.

When these more or less pigmented hairs begin to be pigment free, less pigment-forming substance will be used by the epidermis. This circumstance may give an impulse to the appearance of new chromatoblasts, or throw a greater burden on the chromatoblasts already present. It is not surprising that with increased formative activity the chromatoblasts should gradually increase in size, take on the round form and proliferate.

Regarding the descent or nature of the chromatoblasts van Dorssen observes that in the round and irregular collections the cells sometimes lie so close together that no trace of intercellular space or intercellular material is to be seen. These cells often have assumed a polygonal form owing to pressure and such groups of closely arranged cells surrounded by a connective tissue stroma certainly at the first glance make one think of an epithelial tumour—a carcinoma.

In most rows and groups of cells, however, in the sections stained with van Gieson or with hæmalum one could recognise between the cells a fine fibrillary substance, and on further search it was seen that nearly every pigment cell was separated from the others by this intermediary stuff. We have thus a connective tissue framework, whose alveoli are filled with larger and smaller groups of somewhat large cells, between which there is a fine network of fibrillary substance. *This is the picture of an alveolar sarcoma.*

In submiliary melanomas a distinct increase of the connective tissue is recognisable ; the stroma has proliferated. This increased connective tissue sufficiently explains the usually firm fibroid consistence of the young melanomas. It is a striking fact that neither in the stroma, nor at the periphery, is there any small-celled infiltration.

ÆTIOLOGY.

The chief purpose of the foregoing quotations was to show how deeply divided opinion is, not only with regard to the source and production of melanin in pigmented neoplasms, but also concerning what may be called its manufacture in the normal body.

The first question that may be considered is, what is the nature of the essential cells in the equine tumour ? The answer to the question is difficult, for several reasons. It will probably never be possible to furnish absolute proof as to whether the equine melanoma has its starting point in connection with epithelium or the connective tissue cells of the corium. The primary cells of a neoplasm may be said to cover up their own tracks, and thus make it impossible to recognise with certainty the microscopic point at which the growth began.

In general, what one has to rely upon is a morphological resemblance between the tumour cells and the normal cells of some adjacent tissue or, if there are considerable differences between the neoplasm cell and the normal cells where the growth is supposed to have begun, one may search for intermediate or transitional cells which might suggest the true descent of the tumour in spite of differences between them and the normal cells in size, shape, or other respects. Van Dorssen thought that in the black specks constituting what he termed " melanosis cutis " in grey horses he could, through transitional stages, trace the actual starting point of a melanoma to a normal connective tissue cell in the cutis.

The difficulty of the question is considerably increased by the fact that the appearance of the pigmented cells in the equine melanoma (see Fig. 7) is unlike what one would have expected in either epithelial cells or cells of the connective tissue type. The curious resemblance of the cells after bleaching of the melanin to the

cells of a lipoma from which the fat had been dissolved out has already been mentioned. But that does not help much to decide whether the cells are derived from epithelium or connective tissue, for epithelial cells, notably those of the liver, can assume a similar shape when infiltrated with fat.

Van Dorssen was strengthened in his opinion that the melanomata are built up by cells of the connective tissue type by his failure to recognise such pigmented cells in the corium of the skin of horses of any other colour than grey. He appears to

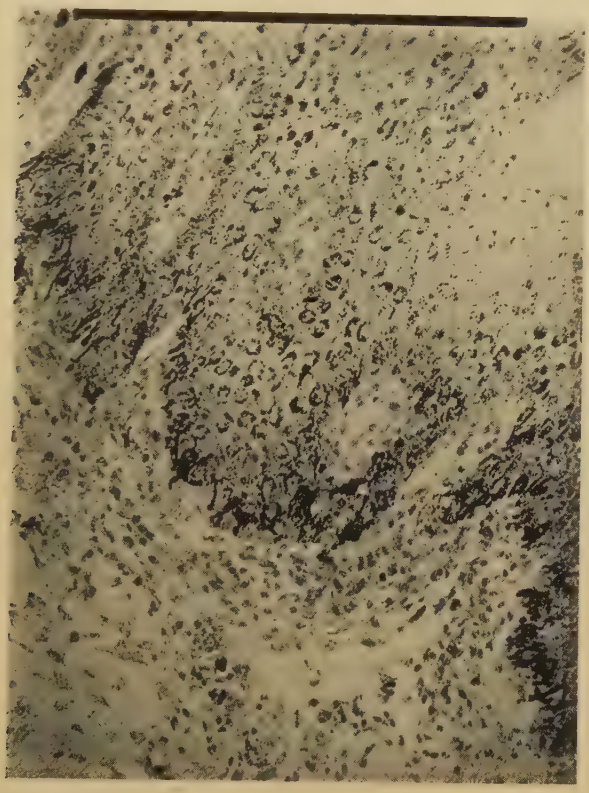

Fig. 8.

have been sceptical about the occurrence of melanomata in such horses and thought it probable that horses of other colours than grey failed to develop melanomata because they lacked the cells from which the melanomata in grey horses take their origin, but I have myself had the opportunity, without any special endeavour,

to examine four such cases. The particulars with regard to these animals were :—

1.	Roan horse.	Metatarsal region.
2.	Bay horse.	Tail.
3.	Chestnut mare.	(Site omitted from the record.)
4.	Bay horse.	Flank.

On the view that the basal cells of the epithelium have the ability to form melanin, it is difficult to understand why both in man and in horses and cattle the neoplasm which undoubtedly takes its origin in the basal cells of the epithelium—the so-called epithelioma—is seldom or never pigmented. I consider myself fortunate in this connection in having had an opportunity to examine two examples of the so-called melanotic carcinoma. The first of these has already been recorded, but the facts may be recalled here. The tumour developed on the tail of an aged bay gelding. It was about the size of a man's fist, had an ulcerating surface, and projected a little above the normal level. It extended for about 1½ inches into the structure of the tail. On section it was found to be pigmented, but not so deeply or so uniformly as is the rule in the equine melanoma. Microscopic examination showed that the growth had the histology of a squamous-celled carcinoma, with large, irregular groups of epithelium and a rather abundant fibrous stroma. Melanin was present in both the epithelial masses and the stroma, and in both positions it was partly in the cells and partly free.

The second tumour was excised during life from near the point of the hip of a bay gelding, it was somewhat smaller than the first, and it was visibly but not deeply pigmented. It also proved to be a carcinoma, with a moderate amount of melanin in both epithelium and stroma.

The fact that requires emphasis regarding these two tumours is that histologically they bore no resemblance to the genuine equine melanoma.

In recording the first of these melanotic carcinomas* it was observed that the rarity of such tumours was explainable on the view that " the formation of melanin is a property not of epithelium but of connective-tissue cells and that the pigmentation of even the normal epidermis is dependent upon the agency of mesoblastic cells which elaborate the melanin and then wander with it into the rete mucosum, where it is taken up by the epithelial cells."

In this connection, it may be observed if horses of any other colour than grey had been exempt from melanotic tumours of the skin the fact would have raised a second question more difficult to answer than the one now being discussed, since there is no reason to think that such exemption exists in any other species or breed of the domesticated animals. The fact that one observer has, so to speak incidentally, met with four cases in horses of other colours than grey, makes it probable that, except in greys, the incidence

**Jl. Comp. Path. & Ther.*, Vol. iii, 1880, p. 140.

of the disease in the equine species is about the same as in the other farm animals.

It is obvious that the tendency to the disease in grey horses is somehow connected with the gradual whitening of the hairs, and the fact suggests that some light might be thrown on the ætiology of the disease by a study of the change in colour which occurs annually in the hairs of animals that acquire a white coat on the onset of winter. This had already been thought of by van Dorssen, but he found that Schwalbe's researches on the subject had shown that in the permanently pigmented pieces of skin pigmented cells are not present in the connective tissue either in winter or summer, and further, that a pure epithelial pigmentation occurs without a trace of pigment in the underlying connective tissue. Schwalbe therefore denied the transport of melanin from the cutis into the epidermis.

The ætiology of the equine melanoma, however, goes behind the descent of the essential cells and requires an answer to the question, What is the true determining factor which by its action endows the normal cell, whether epithelial or connective tissue, with the distinctive characters of the cells of neoplasms ?

Here, fortunately, we have a case in which the possibility of a microparasitic cause may be ruled out; the equine melanoma is perhaps the only malignant neoplasm about which that would be generally admitted at the present time.

There are other accepted or suggested causes of malignant neoplasms in man and animals that may also be rejected because, while they might have been satisfactory if the incidence of melanosis had been uniform in animals of the equine species, they fail entirely to explain the high incidence of the disease in grey horses.

It is impossible to think of any suggested external cause of tumour formation that acts specially on grey horses, as there is nothing special in the nature of their diet, work or environment.

One is thus brought back to the fact that the special liability of grey horses to the disease is connected with the steady whitening of the hairs which appears to reach its greatest intensity after middle life.

In the epithelium of the normal pigmented skin of man and animals there is a steady loss or destruction of melanin. As a rule, it is already unrecognisable in the stratum corneum, and although its destruction there may not be complete, it is in any case cast off in the normal process of desquamation. In the lower animals there is also a great loss of formed melanin in the annual casting of the hairs.

In black, bay, or brown horses the demand which arises in this way for new melanin in the skin and hairs is subject to little variation throughout life, but in grey horses, practically from birth onwards, the demand for pigment begins to diminish, and after middle life rapidly runs down owing to the increasing number of white hairs which no longer require melanin. And it must be remembered that the whitening of the coat is not a process of bleaching due to changes

in the melanin in the hairs, but is the result of an actual cessation of the manufacture or absorption of melanin at the roots of the hairs. There must thus arise in the system of grey horses a considerable disturbance in the provision or manufacture of melanin.

The fact that the most frequent site of the primary equine melanoma is in the skin near the root of the tail is probably connected with the decline in the demand for melanin in the mass of long tail hairs.

The great frequency of the disease in grey horses makes it obvious that in these animals there is an inherited tendency to melanosis. It is now a distinctive character of horses of that colour, and the fact appears to have no parallel in connection with the incidence of any other neoplasm in man or animals. The predisposition may have arisen as a mutation.

The high percentage of grey horses that actually develop melanotic tumours has already been mentioned. It appears to be not improbable that if all grey horses were allowed to die from old age, none of them would escape the development of melanotic tumours. However, the recognition of what may be called a tendency to melanosis in grey horses leaves the cause of the primary melanoma in these animals quite unexplained.

The researches of the present century have indicated that chronic, sustained, or repeated irritation or injury is an ætiological factor in many malignant neoplasms in man and animals, and this fact naturally suggests for consideration the possibility that a similar agency may be at the root of equine melanosis. It is obvious, however, that none of the forms of irritation that have been shown to predispose to or cause other neoplasms can be here incriminated. There is no conceivable external cause of irritation or injury to which the skin of grey horses is specially exposed, but there remains the possibility that there may be an internal or autogenous agent or noxa capable of the same effect. This was suggested by Post as a possible explanation of the origin of melanotic neoplasms in man and animals generally, but what is here required is not a general explanation but one that will fit the case of equine melanosis and will explain the great frequency of the disease in grey horses and its comparative rarity in horses of any other colour.

It appears to be impossible to say whether the whitening of the coat in grey horses is due to a decline in the formation of melanin or an inability of the epithelial cells of the skin and hairs to incorporate it, but van Dorssen suggested that the whitening of the hairs must give an impulse to the formation of new chromatoblasts or necessitate increased activity of those already existing, with the result that they gradually increase in size and form and begin to proliferate. The process would be akin to a compensatory hypertrophy, but there is an immense experience against the view that hypertrophy is likely to be followed by the uncontrolled multiplication of cells which is characteristic of malignant neoplasms.

There is just as little to be said for Post's view that the melanin in the cells of melanotic tumours contains some injurious substances capable of stimulating cells to proliferate.

It must, unfortunately, be admitted that the equine melanoma still remains in the list of malignant neoplasms whose origin is unexplained.

There is still a defect in the evidence regarding the histology of the skin in grey horses during the first five years of life.

Summary.

Melanomatosis is a disease to which all grey horses are specially predisposed. This may have originated as a mutation.

The evidence regarding the nature of the melanoblasts is inconclusive, but the balance is in favour of the view that they are derived from the mesenchyme and not from epithelium. A fact strongly against the latter view is that tumours arising in connection with the epithelium of the skin—the epitheliomata—are usually devoid of pigment and have a histology entirely different from that of the melanomata.

The development of the tumours in grey horses is ætiologically connected with the progressive diminution in the amount of melanin in the hairs, and further evidence is required regarding the histology of the skin in foals and animals under the age of 5 years.

No satisfactory theory regarding the acquirement of neoplastic characteristics by normal melanoblast cells of the horse's skin has yet been presented.

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DESCRIPTIONS OF FIGURES.

FIG. 1.

From a melanoma in an aged grey horse (2848). Stained methylene blue. ×200. The figure shows that at this place about half of the tissue appeared as masses of melanin, the remainder being a connective tissue stroma. Here the connective tissue was not distinctly fibrillated, but at other places it was.

FIG. 2.

From a melanoma in an aged grey mare (2812). Stained methylene blue. ×134. The individual pigmented cells are here recognisable. At some places they form groups without any intercellular material, but elsewhere there is what appears like a fine net-work, which is itself slightly pigmented with melanin. Its appearance suggests a degenerated connective tissue, and it is to a large extent denucleated. In the centre of the figure is a giant cell ; the white dots in this and other cells are nuclei, which, under the microscope showed methylene blue staining.

FIG. 3.

From another part of the same section (2812) as Fig. 2. ×134. It shows the same arrangement of large cells completely filled with melanin, in a delicate slightly-pigmented stroma. At some places the pigmented cells show a distinctly linear arrangement, which suggests that the cells developed in the interstices of the original connective tissue of the place. Cutting off the left hand upper corner there is a strand of ordinary white fibrous connective tissue.

FIG. 4.

From a melanoma in an aged grey horse (3207). ×134. The tumour was on the under side of the tail, near the anus. It shows the same arrangement of melanophores and slightly pigmented stroma as the previous two figures.

FIG. 5.

From same tumour as Fig. 4. ×134. The section was decolourised (in a solution of bichromate of potassium and sulphuric acid) before staining with hæmatoxylin and cosin. It shows at some places groups of small nucleated cells, probably young melanoblasts.

FIG. 6.

From same tumour as Fig. 3. The section was almost completely bleached and afterwards stained with hæmatoxylin and eosin. ×134. It shows the same arrangement of the melanophores in columns, and on the right hand side there are some strands of connective tissue. On the left hand in the upper half of the tumour, and at some other places, there are groups of small nucleated cells apparently at an earlier stage in the growth of the melanoblasts.

FIG. 7.

From same tumour (3207) as Fig. 4. ×263. Decolourised and stained hæmotoxylin and eosin. The figure shows the superficial position of the nuclei in the melanoblasts and the resemblance of these to adipose cells.

FIG. 8.

From same case as Fig. 4. Stained methylene blue. ×200. The section is from part of the intact skin which covered the tumour. It shows a striking abundance of melanophores insinuated between the deep strata of epithelial cells of the skin. They might be interpreted either as carrying melanin to, or removing it from, the epithelium. In the corium there were a few small pigment-carrying cells.

ABSTRACT.

RIFT VALLEY FEVER.

By R. Daubney and J. R. Hudson.

The disease in July, 1930, was causing a heavy mortality in new newly-born lambs on a farm in the Rift Valley. The farm has an altitude of 5,000 to 6,000 feet, and consists of about 30,000 acres of open bush country. It had carried Merino sheep successfully for a number of years. Normally, lambing takes place on this farm in October and November, but on the present occasion that had been changed to July and August, which are usually dry months.

During the latter part of June, before lambing had commenced, there had been a number of abortions in the ewe flocks. At first it was thought these might be due to " blue-tongue," since on two occasions blood taken after ewes had aborted produced blue-tongue on inoculation into susceptible sheep at the laboratory, but other samples similarly connected failed to transmit blue-tongue on inoculation. At the outset the disease appeared to be confined to the lambs of one flock of ewes, and by July 10th 60 had died out of a total of 80 born in the flock. At the time of death the lambs were from three to seven days' old, the majority about three days. The symptoms were listlessness, disinclination to feed and inability to stand. Diarrhœa was not a feature of the condition, and the symptoms were not very definite. Affected lambs rarely survived 24 hours after the first appearance of symptoms.

Coincidently with the appearance of the disease in lambs, there was a marked rise in the mortality rate of ewes on the farm, and the number of abortions increased. Contrary to what was the position with regard to the lamb disease, both mortality and abortion were observed among ewes in practically all the flocks on the farm. The ewes were either found dead without having shown any symptoms, or were observed to be ill for a few hours only before death. In some cases, it was said they had been observed to vomit in the evening and were found dead in the morning.

In sheep observed to be ill, there was a thick muco-purulent nasal discharge, and occasionally the stools consisted of almost pure blood. The ewes were not feeding and were disinclined to rise, but at this stage they had a normal temperature. Ewes that aborted were not visibly ill at the time, with the exception of a few animals which developed septic metritis or peritonitis. A month later, July 27th, the situation had become far more serious, and cases were then occurring in all the flocks of lambing ewes. Abortions among the ewes had almost ceased, but the ewe mortality was now extremely heavy.

A fortnight later the position was much the same. The total mortality in lambs had now reached approximately 3,500, and upwards of 1,200 ewes had died.

By this time it was known from experiments that the disease in the lambs was a hyper-acute form of that in the ewes and that abortion was a common sequel to recovery in pregnant animals.

After the removal of some of the flocks to a farm at a higher altitude (7,000 to 8,500 feet) deaths continued for a further period of five or six days, but from that time onwards no further cases were recorded. It was considered reasonable to suppose that those animals that had died during the

first few days after the movement had been infected before leaving the original farm.

On the lower farm the disease continued for several months, and occasional cases were observed as late as December.

The lesions encountered at a post-mortem examination are very constant, whether naturally or experimentally infected. In adult sheep the lesions at first appear to be quite different from those found in lambs, and this applied more particularly to the liver lesion, but further investigations have shown that the apparent difference depends solely on the acuteness and rapidity of the attack.

The most striking lesion in lambs is extensive necrosis of the liver, which is at first of a focal nature, but in severe cases may affect most of the liver tissue. The necrotic foci appear beneath the capsule as small white spots up to 1 m.m. in diameter, and near these there are commonly some scattered pin-point subcapsular hæmorrhages ; the liver is not enlarged. The necrotic foci are distributed more or less evenly throughout the whole of the organ, but by coalescence they may form a diffuse necrotic lesion.

Such acute liver lesions are rarely seen in adult sheep. In those dying of either the natural or the experimental disease the liver shows innumerable small subcapsular hæmorrhages of pin-head size and reddish-brown in colour. There is usually no increase in the size of the organ, but it may be engorged with blood.

Spleen.—Enlargement of the spleen has been observed only once in 200 post-mortem examinations. The constant lesion is the presence of pin-point to pinhead-size hæmorrhages beneath the capsule. The pulp of the organ is not noticeably affected.

This condition of the spleen is held to be important for diagnosis, as it enables one to exclude Nairobi sheep disease, heartwater and blue-tongue.

Kidneys.—The capsule strips normally and the exposed cortex usually shows a few small hæmorrhages. On section radiating lines of congestion may be seen in both cortex and medulla, but otherwise the tissue appears normal.

Lungs.—These are little changed, but a fairly constant lesion is the presence of small slightly-raised areas on the surface. These are irregularly oval shape, and may be up to an inch in their larger diameter. They are a little paler than the surrounding tissue.

Circulatory System.—Sub-epicardial ecchymoses are common in the region of the coronary grooves on the heart, and fairly extensive sub-endocardial extravasations are almost constant in the left ventricle. Strong congestion of the mesenteric and omental vessels is another characteristic feature of the disease.

Lymphatic System.— The mesenteric lymphatic glands are always enlarged and in most cases there are extensive hæmorrhages in the cortex.

Alimentary Tract.—The mucous membrane of the abomasum may be faintly congested or show pin-point hæmorrhages towards the cardiac end.

There may be catarrh of the mucous membrane of the small intestine, and the ileum is not infrequently the seat of diffuse hyperæmia. The cæcum may show areas of congestion and this may extend to a slight distance into the colon.

In lambs the cæcum may be impacted with a pultaceous mass, and in a few cases there is marked hæmorrhagic enteritis affecting chiefly the

ileum and the large intestine. This may lead to considerable thickening of the mucous membrane in adult sheep. In these cases the colon is impacted with chocolate-coloured fæces, "tarry" in consistence and containing a large amount of blood. It is, however, not considered certain that the tarry enteritis is not due to some complicating infection.

There is a very remarkable tendency to rapid decomposition in the carcases of animals that have died of the disease, and sheep that die in the night are frequently too decomposed for post-mortem examination by 9 o'clock on the following morning.

No macroscopic lesion beyond injection of the meningeal vessels was found in the nervous system.

Ætiology.—The disease is transmissible experimentally by the inoculation of blood or extracts of organs. In sheep so infected, there is a sharp rise of temperature after from 24 to 48 hours. The mortality from experimental infection in adult sheep is less than 20 per cent., and the induced disease differs in no way from that contracted naturally. In young lambs the mortality from experimental inoculation is probably in excess of 95 per cent., and death may occur as early as 36 hours after the inoculation of virus. Recovered animals possess a high degree of immunity, and this made it possible to demonstrate by means of cross immunity tests that the ewe and lamb diseases were identical.

After repeated failure of attempts to isolate some causal bacterium from the liver tissue or from other organs, it was proved that the disease was due to infection with a filterable virus. The virus is present in the blood and in extracts from organs and it passes through the finest laboratory grades of filter. It is resistant and can be kept in cold store for several months in citrate or in O.C.G.

Natural Transmission.—In spite of the fact that susceptible sheep have been housed along with others infected with the virus, infection by contact has never occurred. Such in-contact animals have always been proved susceptible when tested as to their immunity afterwards.

Experiments in the field designed to test the possibility of transmission from ewe to lamb by contact and by feeding with milk broke down owing to the rapidity with which the disease spread to all parts of the farm, including the buildings.

At the laboratory nursing ewes have, on occasions, been inoculated with virus and have reacted and even died without infecting their lambs; conversely, young lambs infected by inoculation have died without infecting their mothers.

Although the disease in the field assumed the proportions of a serious epizootic it failed to spread naturally in the sheep population at the laboratory in spite of close contact and the proved susceptibility of the great majority of the animals. It is thought that the most feasible explanation of this state of affairs is that the disease was indirectly transmitted from animal to animal, and that the necessary transmitting agent was absent from Kabete (where the experiments were conducted).

A field exposure experiment carried out on the farm where the disease first occurred indicated that in all probability transmission is effected by some insect that is sufficiently large to be excluded by an ordinary mosquito net, and which feeds chiefly at night. This hypothesis was supported by certain features in the history of the original outbreak.

The only goat inoculated with the virus gave a reaction identical with that shown by sheep and recovered. No outbreaks have been reported in

goats, although when ewes have been aborting heavily in an infected area reports of abortions in pregnant goats have also been obtained.

Cattle are susceptible to the virus and a natural outbreak of the disease was observed in a dairy herd. This occurred on a farm in the Rift Valley, between 30 and 60 miles from the first outbreak in sheep. There were no sheep on this farm, but sheep and goats belonging to natives' quarters on a neighbouring farm were reported to be aborting in large numbers. About 50 cases of illness were observed in the dairy cows, there were three deaths during the acute attack and a further four deaths from *sequelæ*, such as abortions. The symptoms were not very definite, but there was more or less complete temporary cessation of lactation. Salivation was a common symptom, and at times there was fetid diarrhœa. Few temperatures had been recorded, but one animal that was salivating and purging had a temperature of 107°. Blood from this animal was despatched to the laboratory and used for the inoculation of two bullocks, two sheep known to be immune to the disease, and two sheep susceptible to the disease. The bovines, which had been bred in the Rift Valley, failed to react, as did also the two immune sheep. The susceptible sheep reacted typically, recovered, and subsequently proved immune to a sheep strain of virus. These and other similar experiments proved conclusively that the bovine disease was Rift Valley fever.

The only symptom shown by the experimentally infected cattle, apart from dullness and inappetence, was the presence of blood-stained mucous nasal discharge. The temperature reaction commences on the average about 24 hours later than it does in sheep. The lesions found post-mortem resemble those produced in adult sheep.

During the course of the infection the four Europeans who were engaged in the investigations all developed a dengue-like fever, which was afterwards recognised to be due to infection with this virus.

In every case the onset of the attack was characterised by a very brief period of general malaise, followed rapidly by sharp rigors and headache. The temperature rose to about 103° and the face was brightly flushed. In from three to six hours after the rigors had passed off, pains developed in or near the joints extending from the base of the skull to the extremities. Fever persisted for a period from 12 to 36 hours, and the pains gradually disappeared within about four days. One of the patients had a second temperature reaction accompanied by similar symptoms three days after recovery from the initial attack. He complained of headache and defective vision for some weeks afterwards.

Upon enquiry it was found that almost every native engaged in herding sheep during the original outbreak had been ill for some four days, and had complained of fever accompanied by severe pains. At the time these illnesses were ascribed by the manager to the eating of somewhat decomposed carcases of dead sheep. The manager of the farm where the outbreak in bovines was discovered also contracted the disease, and it was in fact his description of what he considered to be an attack of influenza that made the authors suspect the true nature of the disease occurring in his cattle.

The disease has been inoculated to man and the effects were identical with those experienced by the authors as reported above. Only one horse had been inoculated with the virus and the result was negative. Two pigs similarly inoculated also failed to react in any way.

The authors state that the disease has undoubtedly existed in the Rift Valley for some years, and has, from time to time, been responsible for

extensive losses both in adult sheep and in young lambs. Precise records are not available, and the information obtained from stock-owners indicates that in certain wet years there has been excessive numbers of abortions and a heavy mortality in adult sheep. In other wet years the chief feature has been the mortality in very young lambs. In each case there has been a history of abortion in cattle and a dengue-like fever in man, occurring at the same time. The mortality in lambs was generally attributed to a bacillary infection, probably to *B. coli*, and the mortality in ewes was thought to be due to heartwater—a disease which, before the discovery of Rickettsia, presented a considerable difficulty in diagnosis. The dengue-like fever in man, would in all probability have failed to attract serious attention in view of the fact that in such years the incidence of malaria would be unusually high.

In the experience of the authors, the two chief factors concerned in the natural control of the disease appeared to be the lower susceptibility of adult sheep and the distribution of the vector. The numbers of the transmitting insect appear to be controlled by climate, which operates by limiting its distribution both geographically and seasonally. If lambing is taking place when and where the vector obtains its peak in numbers, heavy mortality among young lambs is to be expected. If, on the other hand, it is possible to arrange that lambing shall fall at a time when the vector is not normally active, or it is possible to lamb at higher altitudes where the vector does not occur, mortality may be greatly reduced.

In the light of these facts, it was found possible to prevent serious outbreaks on the farms in the vicinity of the original outbreak during the following year.

The authors propose the name of " Rift Valley Fever " as a popular alternative to their first suggestion of enzootic hepatitis, which was originally applied to the disease in sheep.

The disease is distinguished from the disease of cattle known under the names " three-days sickness," " stiff sickness," " ephemeral fever," and dengue, by the different symptoms and the different course of the two diseases. Stiff sickness lasts from three to five days, and is followed by a rapid and complete recovery. Affected animals are invariably lame in one or more limbs and may be so badly affected as to be unable to rise ; further, Montgomerie was unable to transmit three-days sickness to sheep, although some 20 sheep were inoculated on various occasions with blood containing the virus.

The true dengue of man is not transmissible by inoculation to cattle, but at the date of the authors' report there appeared to be no records of attempts to infect sheep with that disease.—(*Annual Report, Department of Agriculture, Kenya*, 1930.)

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